# PARASITOLOGY

# PARASITOLOGY

*(Founded by G. H. F. Nuttall, F.R.S.)*

EDITED BY

D. KEILIN and P. TATE

IN CONJUNCTION WITH

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# OBSERVATIONS ON A NEW CERCARIA DEVELOPING IN *MELANOPSIS PRAEMORSA* IN ISRAEL

BY H. ULLMAN

*Department of Parasitology, Hebrew University, Jerusalem*

(With 20 Figures in the Text)

## INTRODUCTION

There have been few investigations on larval trematodes occurring in the Near East: (1) Sonsino (1892) described several species of cercariae from fresh-water molluscs in Egypt; (2) Looss (1894) published similar observations; (3) Khalil (1933 and 1939) established that *Cercari apleurolophocerca* from *Pirenella conica* from Egyptian lagoons, contrary to the descriptions of Sonsino and Looss, has dorso-ventral finfolds and not lateral ones, and that it is the larval form of *Heterophyes heterophyes*; (4) Abd el Azim (1933) demonstrated experimentally that *Cercaria vivax* Sonsino, 1892, is a larva of *Prohemistomum vivax*.

Since we knew that the snail *Melanopsis praemorsa*, found in the Yarkon River, is a host of many forms of cercariae an investigation of these cercariae was undertaken. Snails of this species have been repeatedly collected over a period of 18 months and morphological, statistical and biological facts have been established. At least nine different species of cercariae were found in *M. praemorsa*. In the following pages a cercaria of the *pleurolophocerca* type is described as a new species, *Cercaria orospinosa*. One of the remaining species has been found to be related to *C. vivax* Sonsino. A third species is very close to *C. pusilla* as described by Wesenberg-Lund (1934). A fourth one is a close relative of the cercariaeum named by Sewell *C. indica VI*. The other cercariae seem to represent new species as far as could be concluded from the available literature. Although snails were collected at all seasons for the purpose of study of the material, statistical examinations of infestation rate were performed on a few occasions only. On each of these occasions eighty snails were investigated *by dissection* and the findings were recorded. It is remarkable that the observation of the emergence of the cercariae from the snails in vials reveals only a fraction of the infestation rate.

As shown in Table 1, the infestation rate of *Melanopsis praemorsa* varied in different batches. Only two of the above-mentioned species of cercariae were present every time. Some cercariae were rarely found, but *Cercaria orospinosa* was predominant in every lot examined. During all seasons, a batch of snails collected from the river at random showed infestation with this trematode. Fluctuation of the percentage of snails infected with this cercaria was, however, considerable, from 4 to 35%.

## THE HABITAT OF *MELANOPSIS PRAEMORSA*

*M. praemorsa* was collected from the River Yarkon in the vicinity of the bridge near Petah Tikvah (north of Tel Aviv), about 12 km. from the mouth of the river. This place was chosen because of the constant high rate of cercarial infestation in

snails found there. The snails are abundant in the water on the banks, ploughing through the mud. They are also found attached to dead tree twigs and to the underside of the leaves of floating water plants and on rotting plant material. In this region the river flows at a speed of about 40 cm./sec. and the water is semi-transparent. The dominant water flora consists of *Phragmites communis* on the banks and by *Potamogeton nodosus*, *Myriophyllum spicatum*, *Nuphar luteum*, floating in the river. The relatively high temperature of the water in this region during the cold season of the year probably accounts for the large numbers of this snail found there.

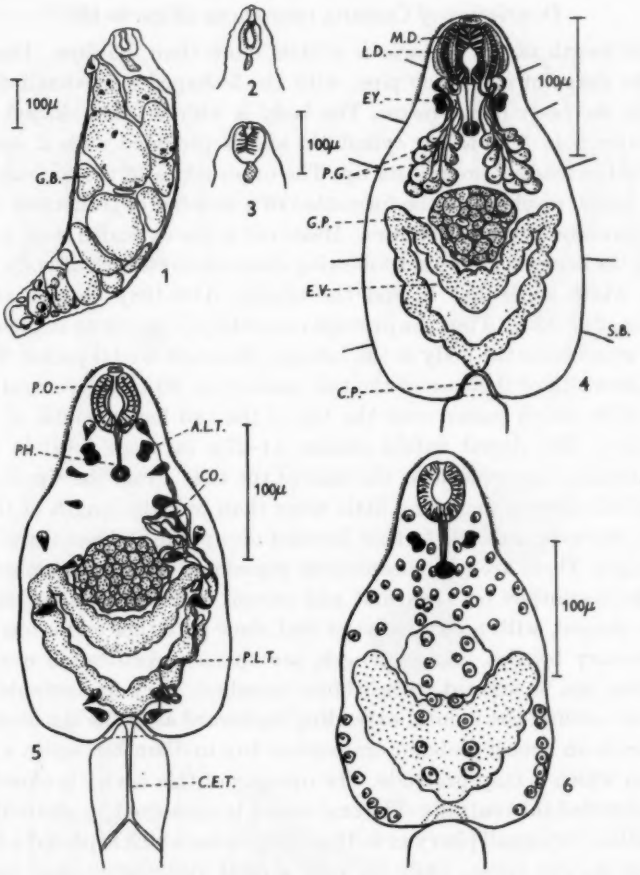
Table 1. *Infestation rate of* Melanopsis praemorsa *with different species of cercariae, established by dissection*

| Species of cercariae | Date of dissection | | | Average |
|---|---|---|---|---|
| | 12. ii. 50 | 8. iii. 51 | 10. iv. 51 | |
| *Cercariae microcotylae* (at least two different species) | 4·9 | 6·0 | 5·6 | 5·5 |
| *C. virgulae* (two different species) | — | 6·0 | — | 2·0 |
| *Cotylocercous cercariae* | 1·2 | 1·2 | — | 0·8 |
| *Xiphidiocercaria* sp. | 2·4 | — | 4·2 | 2·2 |
| *Cercariaeum* sp. | 1·2 | — | 1·4 | 0·8 |
| *Cercaria vivax* (?) | 1·2 | — | — | 0·4 |
| *C. orospinosa* n.sp. | 27·5 | 35·0 | 4·2 | 22·2 |
| Total percentage of infestation by cercariae | 38·4 | 17·7 | 15·0 | 23·7 |

*The redial stage of* Cercaria orospinosa (Figs. 1–3)

About 300 specimens of *Melanopsis praemorsa* were dissected during the whole year, but sporocysts of *Cercaria orospinosa* were never found. Rediae were at times so abundant that their composite volume seemed to be bigger than that of the harbouring organ, the hepatopancreas, in which they develop. In infested snails this organ appeared grey, instead of brown as in non-infected snails. When this organ was exposed and teased the rediae fell out readily. The maximum dimensions of the redia were 825$\mu$ in length and 187$\mu$ in diameter. The average redia was approximately 700$\mu$ long and 170$\mu$ broad. The rediae were white and cylindroid with a slightly roughened surface, without any motor appendages. A small terminal pharynx, 27–29$\mu$ in diameter, opened into a relatively small gut. The length of the gut was usually less than a fourth of the diameter of the pharynx. The existence of the gut directly behind the pharynx could be revealed only by staining, the gut is embedded in a small cluster of tissue. The body cavity of the redia is filled with germ balls and cercariae at various stages of development. The smallest germ ball is a single cell 9$\mu$ in diameter with a relatively large nucleus 2·7$\mu$ in diameter. These germ balls apparently are produced from the internal wall of the posterior extremity of the redia. The maximum number of embryos encountered inside a redia was 24, their size showing a steady increase towards the anterior extremity of the redia. In the anterior half of the redia they gradually assume cercarial character with two black eye spots becoming conspicuous. In the

anterior-most part of the rediae the cercariae may be almost completely developed. They move about vigorously. Each redia has an inconspicuous birthpore directly behind the level of the gut. The maximum number of embryos with distinct cercarial characters contained in the cavity of the redia may be eight. The final




Figs. 1–6. *Cercaria orospinosa*. Fig. 1. Redia with germballs and cercariae in various stages of development. Figs. 2 and 3. Two stages of suctorial movements of the lips and the pharynx of the redia. Fig. 4. Anatomical outline of the anterior extremity of the cercaria, showing penetration glands and ducts, and sensory bristles. Fig. 5. Arrangement of the flame cells and their ducts in the cercaria. Fig. 6. Arrangement of cystogenous glands of the cercaria.

Abbreviations used in all figures: *AC.* acetabulum; *A.L.T.* anterior lateral collecting tube; *CAE.* caecum; *C.E.T.* central excretory tube; *CO.* convolute of collecting tubules; *C.P.* caudal pocket; *C.S.* cephalic spines; *C.S.P.* cuticular spinules; *D.F.* dorsal fin fold; *E.V.* excretory vesicle; *EY.* eye spot; *G.P.* genital primordium; *L.D.* lateral ducts; *LI.* lips; *M.D.* median ducts of penetration glands; *OE.* oesophagus; *OV.* ovary; *P.C.W.* primary cyst wall; *P.G.* penetration glands; *PH.* pharynx; *P.L.T.* posterior lateral excretory tube; *P.O.* penetration organ; *S.B.* sensory bristles; *S.C.W.* secondary cyst wall; *S.O.* scarifying organ; *SOL.* solenocyt; *TE.* testes; *V.F.* ventral finfold.

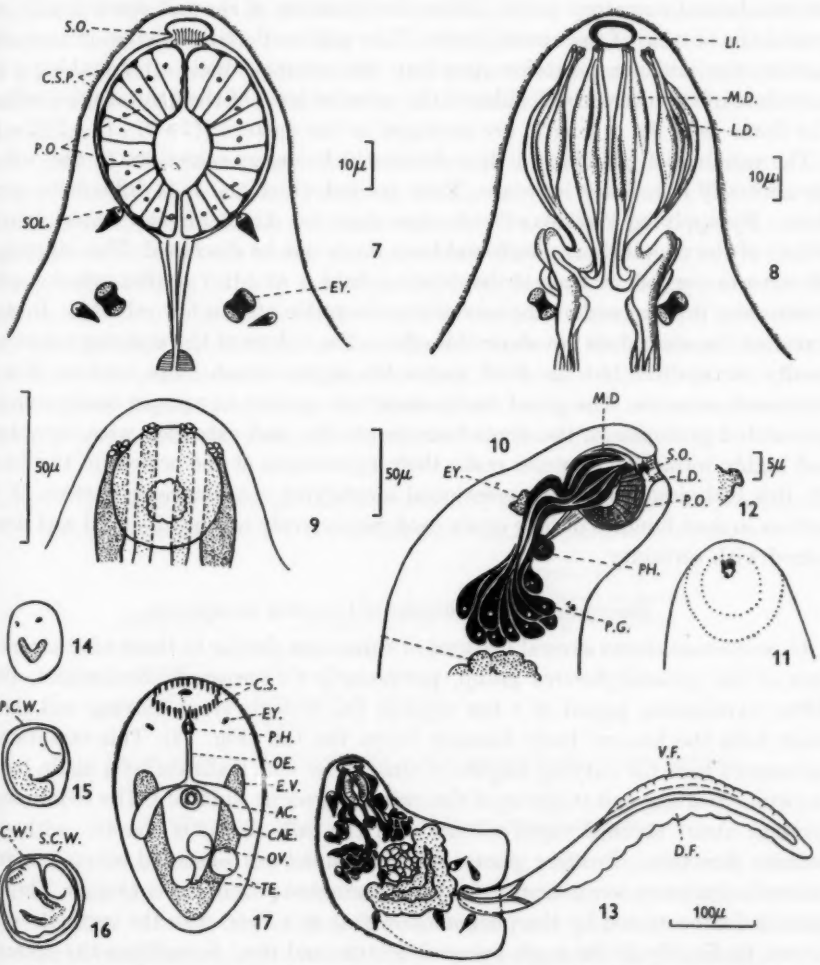
stages of development are attained by the cercariae after leaving the redia. Cercariae sometimes accumulate in the liver tissue or in the mantle cavity. In the latter case they may appear in great numbers attached by their oral suckers when they look like a living fur.

*Description of* Cercaria orospinosa (Figs. 4–13)

The total length of the cercaria is a little more than 0·5 mm. During rest it assumes the shape of a tobacco pipe, with the S-shaped curved tail directed upwards while the body hangs below. The body is whitish, pear-shaped and tapers at its anterior pole. The tail is cylindroid and is provided with a ventral and a dorsal finfold extending around its tip. The outer edges of the finfolds are longer than their bases, resulting in the formation of a number of transverse folds which give the impression of rays or bristles. Measured in the extended state immediately after death the cercaria shows the following dimensions: length of body, 209–319$\mu$, maximum width somewhat behind the middle, 110–198$\mu$; length of tail after decaudation, 242–330$\mu$. Thus the average ratio of body length to tail length is 9/10. The tail is attached to the body at the bottom of a small caudal pocket whose depth is half of the width of the base of the tail, namely *ca.* 20$\mu$. The ventral finfold and that part of it which passes over the tip of the tail has a width of *ca.* 11–19$\mu$ (average 13$\mu$). The dorsal finfold attains 11–27$\mu$ (average 18$\mu$) in width. No cuticular sheath is perceptible at the base of the tail. From the tip of the tail the ventral finfold extends forward a little more than half the length of the tail. The dorsal one, however, extends farther forward occupying at least three-quarters of the tail length. There are two conspicuous pigmented eye-spots, embedded in the body at the borderline between first and second fourths of its length. They are sand-clock shaped, with a narrow waist and show on either end a less pigmented cavity. Sensory bristles, 21$\mu$ in length, are sparsely distributed over the body surface (they can be stained with lithium carmine). Very fine spinules are set in circular rows around the mouth, extending backward as far as the level of the eye spots. There is an almost globular oral sucker 40$\mu$ in diameter, set in a pocket-like cavity from which it may protrude. The opening of this cavity is closed by elastic lips and is directed subventrally. The oral sucker is connected by an undifferentiated ligature with a very small pharynx 9–10$\mu$ in diameter which is placed a little behind the level of the eye spots. Only by very careful observation may this organ be detected but not in every specimen. No further structure of the digestive system is present. The genital primordium appears as a big irregular group of cells approximately 60$\mu$ in diameter in the middle of the body of the cercaria.

A well-developed apparatus for penetrating the host tissues consists of the following four elements: (1) two symmetrically arranged clusters of ten gland cells each, occupying the region between the genital primordium and the eye spots; (2) four bundles of ducts conveying the secretions from these glands to the anterior rim of the oral sucker; (3) a scarifying organ consisting of nine hatchet-shaped blades, each 5$\mu$ in length, set in one row at the dorsal edge of the oral sucker. This organ moves forwards and backwards, widening and narrowing the distances between its blades as it moves; (4) orifices of the penetration gland ducts which

also open on the dorsal edge of the oral sucker. These orifices may be seen only when they are in the proper position and when the physico-chemical condition of the glands make them stand out. They are arranged in four groups, each consisting of a constant number of orifices, namely six in the outer groups and four in the median groups. This arrangement may serve as a valuable characteristic for identification of this cercaria.




Figs. 7–17. *Cercaria orospinosa*. Fig. 7. Oral sucker of the cercaria with the scarifying organ. Fig. 8. Ducts of the penetration glands of the cercaria. Fig. 9. Arrangement of the apertures of the penetration gland ducts. Fig. 10. Anterior extremity of *C. orospinosa*, lateral view, showing the penetration apparatus. Fig. 11. Scarifying organ of *C. orospinosa*, ventral view. Fig. 12. Element of the scarifying organ, lateral view. Fig. 13. General view of cercaria during resting (for natural position, see the text). Fig. 14. Appearance of the cercaria shortly after it ceased to penetrate into the tissues of the intermediate host. Fig. 15. Metacercaria in the state of dissolution of internal structures. Fig. 16. First signs of new anatomic organization of the metacercaria. Fig. 17. Anatomy of the metacercaria.

Stained specimens may show cystogenic cells distributed over the body in four rather indefinite longitudinal rows. The very distinct thick-walled V-shaped excretory bladder is composed of big opaque cells which are filled with granules. It extends forward as far as the anterior level of the genital primordium. The excretory bladder opens into a narrow short tubule entering the base of the tail and forking at about one-fifth to one-sixth of the length of the latter. Thus there are two lateral excretory pores. After the shedding of the tail there is only one pore at the bottom of the caudal pocket. Two main collecting tubules on each side, one anterior and one posterior open into the common duct, after making a big convolute of tubules on both sides of the anterior level of the genital primordium. The flame cells, 7 $\mu$ in length, are arranged in the pattern $[(2+2)+(2+2)]2=16$.

The penetration glands and their ducts which convey secretions to the orifices are normally in an inactive state. They are indistinct and it is difficult to count them. By applying Westblaad's alizarine stain (*ca.* 1:1000 in tap water) *in vivo*, details of the penetration glands and their ducts can be discerned. This staining is effective in very short time if the diluting fluid is at pH 7 (buffer solution after Soerensen), it then renders the secretion perceptible after a few minutes. In dead cercariae the stain does not show this effect. The colour of the staining solution is hardly perceptible, but the fluid makes the organs much more evident than in untreated cercariae. The gland nuclei stand out against an opaque background of granulated protoplasm, the ducts become swollen and extended with secretions, and highly refractive granules make their appearance at the orifices of the ducts. By this technique the above-mentioned identifying characteristic pattern of the orifices in four bundles of four or six each respectively can be observed and determined with certainty.

### *Emergence and behaviour of* Cercaria orospinosa

*C. orospinosa* shows several features of behaviour similar to those of other members of the *pleurolophocerca* group, particularly *C. coronanda* Rothschild, 1938. After a swimming period of a few seconds the cercaria stops moving and slowly sinks with the heavier body hanging below the tail (Fig. 13). This cessation of movement lasts for varying lengths of time, after which the cercaria again begins to swim. Starting and stopping of the swimming occur suddenly. The swimming is brought about through rapid vibration of the tail, and it is chaotic, without a definite direction. Positive phototaxis was, however, observed when cercariae showed a tendency to concentrate at the illuminated part of the container. Thigmotaxis is demonstrated by the phenomenon that in a Petri dish the cercariae come to rest preferably in the angle between bottom and rim. Sometimes the cercariae accumulate at the bottom of the container. In other instances they rise a few centimetres towards the surface of the water, but the ascent is always followed by sinking. In rare instances the cercariae attach themselves to the vertical wall of the container.

The most suitable time for the emergence of *C. orospinosa* from the snail is between 10 a.m. and 1 p.m. Emergence can be substantially enhanced by exposing the snails to natural or artificial light. In this condition, too, the period of emer-

gence is only a few hours in duration, but recurs on the following days sometimes over a period of weeks. In some cases, however, emergence ceases for no obvious reason. The emergence rate is usually at its highest on the first day after the transfer of the snails from the natural habitat to the aquarium. This is illustrated in the following example: 4·2% of *Melanopsis praemorsa* out of 256 specimens shed *Cercaria orospinosa* on the first day after transfer. A week later not more than 1% of the snails shed cercariae. It may be concluded that for purpose of comparison of cercarial emergence the first day after transfer from the natural habitat has to be taken as the measure of infection. It was invariably found that a number of snails which did not shed cercariae in the test-tube proved to be infected on dissection.

In general, the percentage of infected snails increases with age, but is disproportionately large in the youngest specimens (see Table 2). The following explanation of this fact may be considered: below a certain age limit the natural resistance of the snail can barely keep pace with the multiplying parasites, and the young molluscs may rapidly succumb to infestation when rediae and cercariae have increased to such numbers as to destroy essential organs of the host. Above this age limit, the equilibrium between the resistance of the snail and the rate of increase of the parasites shifts to the benefit of the former. The difference between the incidence of infestation in the first and second age-groups of the snails suggests that a proportion of the young snails succumb to the infestation.

Table 2. *On the* 28 *February* 1950, *a total of* 800 *snails were tested for emergence of* Cercaria orospinosa. *These snails were divided into four age-groups*

| Age-group | Length of shell | Percentage of snails infested as shown by emergence |
|---|---|---|
| I | Smaller than 14 mm. | 3·25 |
| II | From 14 to 16 mm. | 2·0 |
| III | From 16 to 18 mm. | 7·0 |
| IV | From 18 to 21 mm. | 10·0 |

On the average 5·5% of the snails in this sample emitted *C. orospinosa*.

### *The metacercarial stage of* Cercaria orospinosa

The data available on the life histories of several pleurolophocercous cercariae indicate that the cercariae penetrate into a cold-blooded water vertebrate where they develop into metacercariae. Experiments were carried out to ascertain whether *C. orospinosa* encysts in a fish. Young specimens of *Gambusia affinis* were used. Snails which had shown a high emergence rate of *Cercaria orospinosa* over several days were put in a small container at room temperature, together with a small number of fish. One of these experiments was carried out in March, as follows: Three young Gambusiae were put into each of five aquaria, together with twenty *Melanopsis praemorsa* which together emitted cercariae at the rate of *ca.* 1000 every day. Only six out of the fifteen fish showed cysts after an exposure lasting 10 days. A total of twelve cysts were found with a maximum of three in one fish.

The rapidity of the penetration of the cercaria into the second intermediate host, as observed by other writers, varies in different species of pleurolophocercous cercariae. Some species of cercariae cast the tail immediately after the first contact with the host, but others retain it until they start to penetrate. The complete process of penetration may take a considerable time as in the case of *Siphodera vinaledwardsii*, as described by Cable & Hunninen (1942), where the cercariae were seen to be completely embedded in the host fish only after 45–50 min. The penetration of *Cercaria orospinosa* into young *Gambusia* is, however, a rapid process and is complete in no more than 1½ min. The cercaria, on coming in contact with the second intermediate host, attaches itself with its sucker and bores into the skin. At times it rests for a while without movement, sometimes wriggling on the spot, and then it renews a vigorous effort to penetrate the skin. The tail assists by driving the cercarial body against the fish, but it is eventually cast off and continues its twisting motions for some time.

At this time, the activity of the penetration glands increases considerably and their morphology becomes as distinct as after applying the alizarine vital stain. The speed of penetration can be partly explained by the activity of lytic enzymes brought into the tissues of the host by protrusion of the oral sucker beyond the circular rim of the mouth, and by scarification of the host skin, produced by the to and fro movement of the spines of the penetration organ. The fine posteriorly directed spinules arranged in transverse rows round the mouth and the adjacent region, may be of some help for anchorage. The cercaria enters the body of the host with leach-like movements and when under the skin it moves rather rapidly until it settles. Eventually it comes to rest for encystment under the skin, in the muscles, or in various other organs. It takes not more than one day for a cyst to be formed around the cercaria, and then the development of the metacercaria begins.

In two cases, the penetration of *Cercaria orospinosa* was observed from the first stage of making contact with the skin of the fish to the encystment in the fin rays of the tail. There the metacercariae were observed to be rotating inside their cysts for 10 days. The rotation then ceased and soon the cysts underwent a slow process of dissolution, eventually being completely resorbed. The phenomenon of resorption of the metacercariae is regarded as a proof that *Gambusia* is unsuitable as an intermediate host.

Tadpoles of *Bufo viridis* were chosen for the next trial which proved successful. In contrast to *Gambusia*, tadpoles showed no resistance to the establishment of cysts of *Cercaria orospinosa* and their development to the metacercarial stage. The infestation of the tadpoles by the cysts may be so intense as to result in the death of the host.

Experiments with tadpoles were carried out as follows: ten tadpoles, *ca.* 15 mm. in length, were exposed for 8 days in a small glass jar to numerous *C. orospinosae* emerging from thirteen snails as used in the previously mentioned experiments. Six tadpoles showed from ten to forty-eight cysts each and four tadpoles died, one of them, *ca.* 17 mm. long, clearly the victim of infestation, no less than 400 cysts were disseminated all over its body. The cysts were particularly concentrated in the tissues around the respiratory and digestive organs.

The tadpole is apparently one of the principal second intermediate hosts of *C. orospinosa* in the natural habitat of the Yarkon River or possibly the only one.

The newly-formed cyst is oval with a transparent wall. It grows slightly during the first week of its development. In one tadpole the cysts had a diameter of 200–230 $\mu$ on the eleventh day after infection. The reaction of the host tissues soon results in the formation of an additional wall around the metacercaria. In pigmented tadpoles, the site of the cysts was marked by its whitish appearance, no pigment being produced by the host tissue surrounding the cyst. (In *Gambusia*, too, no pigment formation was perceptible at this stage.) The tadpoles which survive the infection may develop normally, and the metamorphosis often proceeds normally. Some heavily infected tadpoles, however, do not metamorphose and remain without change for months.

*Morphology of the metacercaria* (Figs. 14–17)

The cercaria established in the tissues of the tadpole is bent upon itself. It may be seen revolving in the cyst for some days whilst the cyst wall proper becomes separated from the host tissue wall. In the first days after encystment, the dark U-shaped bladder and the two dark eye spots appear as prominent features. Soon a stage of metamorphosis supervenes and minute vesicles and granules replace the internal structures. The penetration apparatus, consisting of glands and spines, disappears. The first row of spinules round the mouth grows rapidly and becomes prominent as compared with the rest of the spinules covering the anterior third of the body. Ten days after infective exposure the metacercaria is formed. Around its mouth there is a row of opaque spines consisting of 26 units, each 13 $\mu$ long and 2 $\mu$ wide at the base. The metacercaria has an oval shape and is *ca.* 400 $\mu$ long and *ca.* 200 $\mu$ wide. The oral sucker is transversally oval with a transverse diameter of *ca.* 100 $\mu$. The pharynx is situated about 30 $\mu$ behind the mouth and its diameter is *ca.* 16 $\mu$. The oesophagus is *ca.* 80 $\mu$ in length and bifurcates into two caeca which extend from the middle of the body to the borderline between the second and hindermost thirds of the body. The acetabulum is *ca.* 40 $\mu$ in diameter and lies in the centre of the body. The V-shaped excretory vesicle extends over two-thirds of the body length. In the posterior half of the body between the branches of the bladder and farther backwards, partly overlapped by the bladder, there are three clusters of cells, each having a diameter of about 45 $\mu$. Apparently the anterior one is the primordium of an ovary and the remaining two clusters are the primordia of the testes. No other genital structures are visible at this stage. Two small eye spots (5 $\mu \times$ 11 $\mu$) with their longer axes oblique to the body axis, lie at the same level as the pharynx.

*Experiments to establish the final host of* Cercaria orospinosa

In order to establish the final host of *C. orospinosa* infection of several vertebrates was attempted.

*Dogs.* Fully developed metacercariae were fed to two 3-weeks-old puppies in the form of chopped infested tadpoles mixed with milk. In one case a total of *ca.* 60 cysts of maximum age of 53 days were given. In the second trial a total of

*ca.* 100 cysts of a maximum age of 34 days were fed. After 4 and 8 days respectively, the dogs were sacrificed and the scrapings of the mucosa of the whole intestine were examined microscopically. No trematodes could be found.

*Mice.* Two adult white mice were each fed with about 100 cysts of maximum age of 48 days, in bran. Two days later the mice were sacrificed. Here, too, no trematodes could be found in the scrapings of their intestines.

*Chick.* A 4-day-old chick was fed by a pipette a chopped tadpole containing 40 *ca.* 6-week-old metacercariae. Dissection, 4 days later, revealed no worms in the intestines.

*Eel.* A *ca.* 10 cm. long young eel (*Anguilla anguilla*) caught in a marsh was put in a jar in which were placed a few small pieces of a tadpole infected some 6 weeks before, containing about a dozen of metacercariae. Four days later three living metacercariae were found in its intestine. These were similar to metacercariae found in cysts, but their dimensions were larger: length 0·8 mm., width 0·2 mm., acetabulum 0·05 mm., oral sucker 0·14 mm. wide.

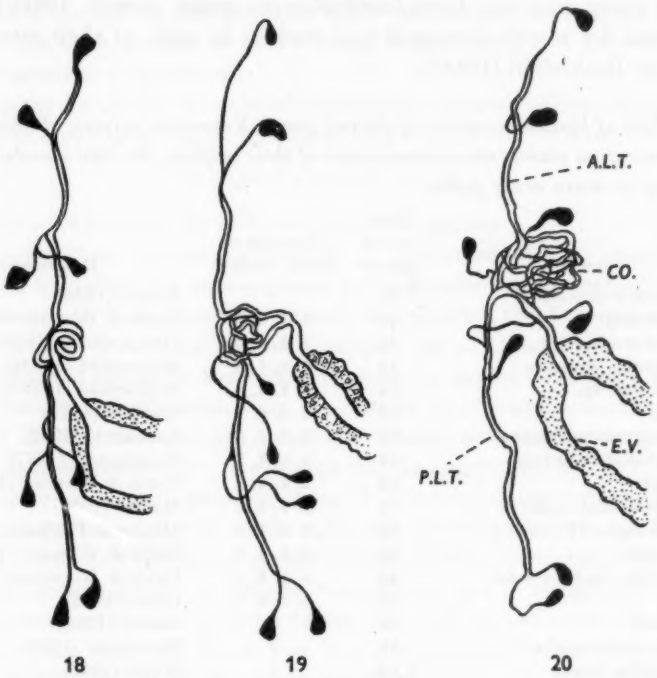
It is noteworthy that ten up to 40 cm. long eels taken in the same river where *Cercaria orospinosa* is common, and at a time when the tadpoles were plentiful, showed no trematode infection.

The conclusion reached from these experiments was that dog, mouse or chick are unsuitable as definitive hosts for *C. orospinosa*. The eel is probably also an unsuitable host, since during 4 days the development was too slow as compared with the development of other species of the *Opisthorchoidea* to which *Cercaria orospinosa* belongs. The surviving of the metacercaria in the eel suggests, however, that this host is probably phylogenetically related to the true host, i.e. another fish. It should be remembered that MacFarlane (1945) found the adult of a related species, *Telogaster opisthorchis*, in an artificially infected New Zealand eel.

### *Taxonomy of* Cercaria orospinosa (Figs. 18–20)

*C. orospinosa* belongs to the *Pleurolophocerca* group of cercariae which was established by Sewell (1922) for cercariae showing a variety of peculiar features, among which, the protrusible oral 'penetrating organ' instead of an oral sucker, long tail provided with cuticular fins, praepharynx and pharynx as the only constant element of the intestinal tract. This group contains cercariae which are very similar, but for purely taxonomic purposes it may conveniently be divided into several subdivisions according to the shape of the tail fins. Martin (1950) suggested four such subdivisions, but additional subdivisions may be differentiated. In the present state of knowledge the limits between these subdivisions seem to be quite distinct, and it would be an advantage to use them as taxonomic units. Consequently, it appears practicable to give separate names to these divisions, whereby they shall be regarded as 'artificial' genera. *Cercaria orospinosa* has one long dorsal and one short ventral tail fin and can serve as the type of a subdivision for which the name *Velocercaria* is proposed. This group has the following characteristics: oral sucker in the shape of a globular penetration organ; weakly developed acetabulum may be present; a small pharynx at a distance from the penetration organ usually present; intestinal caeca absent in most species; a pair of eye spots

between the levels of the penetration organ and the pharynx usually present; no lateral suctorial pockets; tail longer than the body, provided with one longer dorsal and one shorter ventral cuticular fin which may be continuous around the tip of the tail; no distinct lateral tail fins; development in rediae which possess a small intestine and lack locomotor appendages.




Figs. 18–20. Diagrams of flame cell patterns.

Fig. 18. *Cercaria coronanda*. Fig. 19. Cercaria of *Telogaster opisthorchis*.
Fig. 20. *Cercaria orospinosa*.

The art. genus *Velocercaria* belongs, as a part of the *Pleurolophocerca* group, to the superfamily Opisthorchoidea Faust, 1929. It is rather homogeneous and the species which comprise it may be distinguished from one another mainly according to the following character: size, presence or absence of an acetabulum, presence or absence of the intestine, number of penetration glands, arrangement of the orifices of their ducts, flame cell pattern, pigmentation. Presence or absence of the acetabulum which served to the earlier investigators of cercariae as one of the main group-characters, can no longer be applied as a taxonomic criterion in the *Pleurolophocerca* group. This organ not only shows various stages of reduction in different species of cercariae, but it may appear in early stages and disappear during the development of some cercariae, as shown by Dubois (1929, p. 35) in his '*Cercaria lophocerca*'. Similarly, the presence of intestinal caeca should not be regarded as conflicting with the definition of the *Pleurolophocerca* group. This organ occurs in some typical heterophyid cercariae, as for instance in the cercaria of *Apophallus*

*venustus* (acc. to Cameron, 1937) and *Metagonimoides* sp. (acc. to Ingles, 1935), while it is absent in most allied species.

On the other hand, the presence of a well-developed oral sucker instead of 'penetration organ' seems to prevent the inclusion of a cercaria in the *Pleurolophocerca* group. The present author cannot agree, therefore, to unite the *Pleurolophocerca* group with the *Parapleurolophocerca* group (Sewell, 1922) which are characterized by a well-developed oral sucker, in spite of their similarity, as proposed by Rothschild (1938*b*).

Table 3. *List of known cercariae of the art. genus* Velocerca n.nom. *showing number of penetration glands and arrangement of their orifices, the total number of glands serving as main order factor*

| *Cercaria* | Total no. of glands | Formula of gland orifices | References |
|---|---|---|---|
| of *Euryhelmis monorchis* | ? 12 | ? | Ameel (1938) |
| *constrictovesica* | 14 | 3, 4, 4, 3 | Cable & Wheeler (1939) |
| of *Clonorchis sinensis* | 14 | 3, 4, 4, 3 | Hsü & Chow (1939) |
| of *Cryptocotyle jejunum* | 14 | 3, 4, 4, 3 | Rothschild (1938*b*) |
| of *Cryptocotyle* sp. | 14 | 3, 4, 4, 3 | Rothschild (1938*b*) |
| *floridensis* | 14 | 3, 4, 4, 3 | MacCoy (1929) |
| of *Metagonimus yokogawai* | 14 | 3, 4, 4, 3 | Yamaguti (1933) |
| of *Metorchis intermedius* | 14 | 3, 4, 4, 3 | Heinemann (1937) |
| *opacocorpa* | 14 | 3, 4, 4, 3 | Cable & Wheeler (1939) |
| of *Phocitremoides ovale* | 14 | 3, 4, 4, 3 | Martin (1950) |
| *pleurolophocerca* II | 14 | 3, 4, 4, 3 | Maxon & Pequegnat (1949) |
| *semicarinata* | 14 | 3, 4, 4, 3 | Cable & Wheeler (1939) |
| of *Siphodera vinaledwardsi* | 14 | 3, 4, 4, 3 | Cable & Hunninen (1942) |
| *vogeli* | 14 | 3, 4, 4, 3 | Cable (1938) |
| *nicobarica* I | 14 | ? | Sewell (1931) |
| of *Pseudexorchis major* | 14 | ? | Yamaguti (1938) |
| of *Apophallus brevis* | ? 14 | ? | Miller (1946) |
| of *A. venustus* | 16 | 4, 4, 4, 4 | Cameron (1937) |
| of *Metorchis conjunctus* | 16 | 3, 5, 5, 3 | Cameron (1944) |
| sp. from *Oncomelania hupensis* | 16 | 4, 4, 4, 4 | Kuntz (1952) |
| of *Cryptocotyle lingua* | 18 | 4, 5, 5, 4 | Stunkard (1930) |
| *coronanda* | 20 | 4, 6, 6, 4 | Rothschild (1938*b*) |
| of *Opisthorchis felineus* | 20 | 4, 6, 6, 4 | Vogel (1934) |
| *orospinosa* | 20 | 6, 4, 4, 6 | New species |
| of *Telogaster opisthorchis* | 23–26 | ? 6, 6, 6, 6 | MacFarlane (1945) |

The following cercariae probably belong to the art. genus *Velocercaria*; this, however, cannot be ascertained owing to inadequate original descriptions: *Cercaria cordata* of Faust, 1924; *C. elegans* of Bregenzer, 1916; *C. lophocerca* of Dubois, 1929; *C. lophocerca* of Lebour, 1911; *C. lophocerca* of Wesenberg-Lund, 1934; *C. pleurolophocerca* of Hussey, 1941; *Cercaria* of *Metagonimoides* sp. of Ingles, 1935.

Besides, there is a number of cercariae which belong to the *Pleurolophocerca* group, the description of which is inadequate, thus, their exact classification is impossible. Some of them may be included in the artificial genus *Velocercaria* when the details of their anatomy will be better known.

As may be seen in the list of species of the gen. *Velocerca* (Table 3) *Cercaria orospinosa* has to be differentiated from cercariae having twenty penetration gland cells, namely, from the cercaria of *Opisthorchis felineus* and from *Cercaria coronanda*. The main difference is in the arrangement of the four bundles of orifices of the gland ducts. In *C. orospinosa* every median bundle consists of four units while the lateral ones consist of six orifices each; in both other species the arrangement is reverse, i.e. the formula of the orifices is: 6, 4, 4, 6 in *C. orospinosa* and 4, 6, 6, 4 in *C. coronanda* and cercaria of *Opisthorchis*.

On the other hand, the cercaria of *Opisthorchis felineus* differs markedly from both *Cercaria orospinosa* and *C. coronanda* by its flame cell pattern:

$$[5+(5+5+5+5)]\times 2=50,$$

as against $(2+2+2+2)2=16$ with the latter two species.

The morphological differences between *C. coronanda* and *C. orospinosa* are strengthened by differences between their metacercariae: the V-shaped bladder of the metacercaria of *C. orospinosa* contrasts sharply with the Y-shaped one of the metacercaria of *C. coronanda* and mace-shaped one of *Opisthorchis felineus*; the wreath of cephalic spines in *Metacercaria coronanda* consists of fifteen, in *M. orospinosa* of twenty-six units while there are none in the metacercaria of *Opisthorchis felineus*. It appears thus justified to regard *Cercaria orospinosa* as a new species.

The taxonomic position of *C. orospinosa* may be determined on the morphology of its metacercaria which resembles those of *Telogaster opisthorchis* and *Cercaria coronanda*. Both were assigned to the subfamily Cryptogoniminae, the former by Rothschild (1940) and the latter by MacFarlane (1945). *C. orospinosa* belongs to the same taxonomic group. It may be pointed out that *C. orospinosa* shares the flame-cell number (16) with the latter two species, and with respect to the arrangement of the flame cells and the convolute of the excretory tubules it occupies an intermediate position. In *C. coronanda* four flame cells are located anteriorly and four posteriorly, i.e. they show a symmetrical arrangement with respect to the convolute (Fig. 18); in the cercaria of *Telogaster opisthorchis* (Fig. 19) two flame cells lie anterior to the convolute and six posterior to it; in *C. orospinosa* (Fig. 20) the distribution is three flame cells anteriorly and five posteriorly to the convolute.

## SUMMARY

1. An account on the cercariae found in *Melanopsis praemorsa* common in the River Yarkon (Israel) is given.

2. A new species of *Cercaria* of the *Pleurolophocerca* group, *Cercaria orospinosa*, is described.

3. The development of *C. orospinosa* to the stage of metacercaria in the tadpole of *Bufo viridis* shows its affinity to the subfamily Cryptogoniminae (Heterophydae).

4. An artificial genus *Velocercaria* is proposed to comprise cercariae which have common morphology with *Cercaria orospinosa* and are characterized by the same type of tail-fins.

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# A STUDY OF *TRYPANOSOMA CRUZI* WITH THE ELECTRON MICROSCOPE

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(With Plates I–V)

## INTRODUCTION

The present paper will give the first and preliminary results of an attempt to study the finer structure of *Trypanosoma cruzi* with the electron microscope. We have examined the flagellate in all the forms in which it appears during cultivation in artificial media or in tissue cultures with the exception of the intracellular forms which have not as yet been obtained in satisfactory preparations.

This is not the first attempt to study protozoans by electron microscopy. Emmel, Jacob & Goelz (1942) and Emmel, Goelz & Jacob (1942) studied sporozoites of *Plasmodium falciparum* and *P. vivax*, obtained from the salivary glands of mosquitoes. They fixed the sporozoites simply by drying them on the coated screens. In these preparations they observed longitudinal striations in a strong periplast which were denser at one extremity than at the other, so that they distinguish an anterior and posterior part of the organism. They considered the striation as a differentiation of the cytoplasm, comparable with the myonema or fibrils of other protozoa, and related them to the movements of the parasites.

Brown (1945) studied the flagella of various flagellates, and his observations have been confirmed by Kraneveld, Houwink & Keidel (1951). In *Euglena*, *Astasia*, *Achromonas* and *Chilomonas* Brown reported all of the flagella to be of uniform diameter throughout their entire length, and to consist of a denser axonema and a less dense sheath, surrounding it. The axonema was found to be composed of two fibres of approximately the same size. The sheath had the form of a coiled helix. Brown describes in addition a number of fine cilia-like branches on some flagella and refers to them as 'flimmer'. The presence of these so-called 'flimmer' was not confirmed in a subsequent study of *Euglena gracilis* made by Saxe (1947). In a brief note this author also mentions a 'banded pattern' in the pellicle of the organism.

## MATERIAL AND TECHNIQUE

The trypanosome used in the present study was derived from a blood agar culture (NNN medium)†. Preliminary to making the electron microscope screens, approximately 0·5 ml. of culture medium, rich in crithidia forms, and containing also metacyclic forms was washed with Earle's solution and centrifuged for 2 min. at approximately 1000 r.p.m. After centrifugation the supernatant fluid was removed and replaced by new saline solution. The procedure was repeated 2 to 3 times in

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† We are greatly obliged to Prof. M. Kahn for this particular strain.

order to free the trypanosome suspension from the proteins of the culture medium. After washing, the trypanosomes were fixed by adding about 0·1 ml. of 2% osmic acid to the suspension. Fixation was continued for times varying from 10 min. to 24 hr. and was followed by washing with distilled water performed in the same manner as before with salt solution. After two to three washings, the liquid containing the fixed trypanosomes was put on film covered screens* and allowed to dry in dust-protected dishes.

The tissue forms of the trypanosome were derived from cultures of embryonic chicken brain and spleen which had been inoculated with organisms of the same strain. The infected tissue explants were maintained in hanging-drop cultures by the methods described previously (Meyer & Xavier de Oliveira, 1948). When they showed many parasites, they were transferred to cover-glasses coated with formvar films and cultivated here for some days as lying-drop cultures.

When the preparations showed parasites and parasite-containing cells attached to the film-coated surface, the clot and nutrient were gently removed and the remaining cells were placed in vapors of osmic acid for fixation. Excessive washing both before and after fixation was avoided so that the loosely attached parasites might not be removed. Subsequently the area of the film supporting the parasites was peeled from the glass and transferred to the E.M. screens for observation (see Porter, Claude & Fullam, 1945).

## OBSERVATIONS

The blood agar forms show in the phase-contrast microscope the well-known features which characterize this group of organisms (Pl. I, fig. 1). The electron micrographs of this form show most of the same features but of course in greater detail (Pl. I, fig. 2). The body of the trypanosome is usually set off from the flagellum by an abrupt decrease in diameter. It shows a transparent margin and a dark central region which is unfortunately too dense for electron penetration (50 kV. R.C.A. Model E.M.U. 2A), and so no information regarding the structure of the endoplasm and nucleus can be obtained from such preparations. The only component of the endoplasm which is distinctly defined is a spherical body of variable size which reduces the osmic acid more actively than other components and so appears extremely dense.

The flagellum of the blood-agar forms generally shows two portions, which may be referred to as proximal and distal. The proximal portion is the larger in diameter and consists in part of an extension of the body of the protozoan. The axoneme of the flagellum can also be seen in this part and it alone extends into the distal portion. A sheath relatively transparent to electrons is especially evident over the distal end, but covers as well the rest of the flagellum. There are frequently a number of dense particles attached to the extreme of the flagellum tip (Pl. I, fig. 2).

The adult trypanosomes (Pl. I, fig. 3), i.e. the flagellated forms which were obtained by cultivation in tissue cultures are considerably smaller than the blood-agar forms. Their flagella appear shorter and consist only of that portion which is

* These films were floated off standard '1 × 3' glass microscope slides which had been coated by dipping in a 0·5% solution of Parlodian in butyl acetate.

distal in the blood-agar forms. The entire flagellum is covered by a fine sheath which can be followed along the whole body of the trypanosome. The body portion here, as in the blood-agar forms, is too dense for very successful microscopy, and shows little detail apart from extremely dense granules. It thins out along the margin which contains the axoneme to form with the latter the characteristic undulating membrane. Close to the posterior tip of the body it is possible to discern the outline of a spherical body which is taken to be the blepharoplast. In some cases it appears vacuolar and is traversed by a few dense fibres (Pl. IV, fig. 16, *b*).

Beside the grosser features of the trypanosome, the electron micrographs show a number of details of fine structure. For example, the body and flagellum of the parasite is seen to be bounded by a distinct membrane (Pl. II, figs. 5 and 6, *m*) 10 m$\mu$ thick (Pl. IV, fig. 14) and this thickness appears to be the same over all parts of the organism. The continuity of the membrane is especially evident in the tissue forms (Pl. I, fig. 3). Unlike the fibrillar components of the cytoplasm which lie just within the membrane, it persists after extended osmic acid fixation (Pl. II, fig. 5).

The sub-pellicle fibres or striations just mentioned are a striking feature of the blood-agar forms. They are present over the entire body of the trypanosome and its extension into the proximal portion of the flagellum. At the posterior tip of the body they are few in number, but by successive ramifications they increase to fill the larger circumference of the body (Pl. II, figs. 4, 8). In some cases they are arranged spirally about the body portion of the flagellate (see also Kraneveld *et al.* 1951) (Pl. II, fig. 4). The trypanosomes show them especially well after osmic-acid fixation. In the tissue forms the striations although present are less evident (Pl. I, fig. 3).

The individual fibres in the blood-agar forms are about 50 m$\mu$ in diameter and essentially uniform in this respect in all parts of the organism. In dried preparations they extend above the general surface and so appear to represent strands of condensed material (Pl. II, fig. 7). They show density variations which remind one of the cross-bandings found in many protein fibres (Pl. II, fig. 6, *s*). These periodic densities are not, however, sufficiently well resolved in these micrographs to show whether they are definite bands, or whether they arise from the presence of spirally organized component fibrils. In a crude test of their composition the fixed trypanosomes were subjected to tryptic digestion. Thirty minutes was sufficient to remove them. They were similarly destroyed by prolonged exposure of the parasites to osmic acid fixation (Pl. II, fig. 5).

As indicated above, most of the other components of the cytoplasm are hidden by the overall density of the cell body. The single exceptions are some dense granules which appear in the phase contrast image as refractile vacuoles, probably of fat. These vary greatly in size. They occur not only in the body of the trypanosome but also in its extension into the proximal portion of the flagellum (Pl. III, fig. 9). They take up relatively much more osmium than any other component of the cytoplasm and they are fixed in a rigid spherical form by the osmic acid (Pl. II, fig. 7 and Pl. III, fig. 9) as are fat bodies of tissue cells (Porter, 1953). Formaldehyde does not seem to fix them, for after such treatment they burst in

the vacuum of the microscope. The content of these vacuoles may be removed with xylene after only a very brief fixation (3 min.) with osmic acid (Pl. III, fig. 10).

The flagellum in the blood-agar crithidia forms which extends anteriorly from the body has two main components. One of these is a small continuation of the body portion; the other is the axoneme. The cytoplasm of the former, as pointed out above, may show dense lipid granules (Pl. III, fig. 9) and invariably contains the submembrane array of parallel fibres or striae (Pl. III, fig. 11). Neither of these differs noticeably from the same components in the body portion of the trypanosome.

There is some evidence that these two components of the flagellum, the axoneme and the body extension have separate sheaths or membranes from their point of origin or insertion on the body of the cell (Pl. III, fig. 12). If this is true, they normally adhere to form the proximal section of the flagellum. Abnormal living forms have been seen where they are separate.

The axoneme itself is a distinctive and rather precisely formed element of the trypanosome. Its relation to the rest of the organism varies considerably. For example, in what is known as the crithidia form, it appears to end at a point anterior to the nucleus, presumably in the blepharoplast (Pl. I, fig. 2). In metacyclic culture form, it can be followed nearly to the posterior tip of the body (Pl. IV, fig. 13), as in the adult tissue forms where it is prominent along the entire length of the organism (Pl. IV, fig. 16).

The axoneme is prominent because of its density and precise morphology. As near as can be discerned from the micrographs, it consists of a bundle of small component fibrils embedded in a matrix (Pl. IV, fig. 14). The exact number of fibrils is difficult to determine because of the overlap, but there appear to be about nine if allowance is made for those hidden by the six on the upper surface. The fibrils which seem to vary somewhat in density and diameter among themselves are uniform along their length. Those measured are *ca.* 700 A. in width. No definite evidence of periodicity has been observed (Pl. I, figs. 2, 3; Pl. III, fig. 11, Pl. IV, figs. 14, 15).

As with the fine striation of the body, the fibrils are destroyed by trypsin and by extended fixation in osmic acid (Pl. III, fig. 12).

## DISCUSSION

The greater part of the internal structure of the trypanosome was not revealed because it is too thick and too dense for electron penetration. Thus we are no better informed concerning the morphology of the nucleus and associated endoplasma. Of the several methods tried to expose these components, such as tryptic digestion and prolonged fixation in osmic acid, none was effective. It is possible that after fixation, thin sectioning and a more penetrating electron beam* will provide information regarding this part of the cell.

The refractile granules visible in phase contrast and depicted as extremely dense spheres in electron micrographs are quite evidently lipid granules or fat bodies. Their osmophilia and their solubility in fat solvent affirms their lipid nature.

* A few micrographs taken with the Philips electron microscope using 80 and 100 kV. showed significantly more internal structure.

In both respects they are identical with the lipid granules of tissue cells grown in culture. The tendency of the blood agar crithidia forms to deposit fat was noted earlier by Doflein (1929).

One of the more interesting morphological features shown by these micrographs are the fibres which are closely packed in parallel array just beneath the membrane or pellicle of the body. Light microscopy of very large trypanosomes such as *Trypanosoma percae* Brumpt, reveals structures of the same order (Wenyon, 1926), and these have been likened to the myonemes of some of the large protozoans (Wenyon, 1926). The twisting and wriggling motions of the body of these blood-agar forms of *T. cruzi* would appear to require a contractile mechanism, but heretofore nothing of the sort has been clearly demonstrated.*

There is no evidence to suggest that the fibres might be artifacts. To the contrary, their orderly arrangement indicates that they represent similar structures in the living unit. That they disappear under the action of trypsin and prolonged fixation with osmic acid suggests a protein composition. As to function nothing is known, but it is reasonable to suppose that the fibres are contractile and are responsible for the tortuous motions of the blood-agar forms.

The little fine structure apparent in these fibres invites further study. As pointed out above, there are density variations in the fibres which remind one of those encountered in protein fibres, but are not as uniformly spaced or as striking. In places, the major fibre looks as though it might be composed of finer fibrils arranged in a tight coil or spiral.

The picture of the flagellum shown by these micrographs has some interest, especially with regard to the morphological basis for flagellar motion. As characteristic for the group these blood-agar forms sometimes show a rapid flagellar vibration which draws the body of the organism through the supporting medium. This indicates that the beat or vibration must have its origin at the anterior end of the flagellum. The slender extended nature of the structure makes this more or less independent action of the flagellum seem the more remarkable and poses the often repeated question of what internal structure the flagellum had to account for this motion. The problem is ideal for electron microscopy, and has already been given some study by Brown (1945). There are, however, several points of dissimilarity between his observations and those presented above, possibly because of the different materials used.

It is obvious in *T. cruzi* that the axonema is the important morphological unit of the flagellum. The axoneme and the sheath are the only structures consistently present out in the tip, and so it is reasonable to consider the axonema as the main functional unit in the wave motion of the flagellum. It is composed of several longitudinal fibre-like densities which appear to be organized in much the same manner in all the forms studied.† Presumably this organization is important to the

* In a more recent study Lofgren (1950) describes some electron micrographs of *Leishmania tropica* which show a fine longitudinal striation of the body, and longitudinal filaments in the flagellum.

† This longitudinal organization of filaments is also found in sperm tails (Grigg & Hodge, 1949) spermatozoids of ferns (Manton & Clarke, 1951) and in the cilia of all cells thus far studied (Sedar, Beams & Janney, 1952; Fawcett & Porter, 1952).

functioning of the axoneme. In abnormal forms, obtained in tissue cultures, where the flagellum was detached from the body, it has been seen *in vivo* that the free-waving flagellum is contractile. To have the bundle-like structure perform flagellar motions, it is only necessary to make the fibres alternately contracting out of phase. According to this concept, waves of contraction originating at the tip first on one side and then on the other, would pass down the flagellum and give it the typical spiral motion. The trigger mechanism which might start the contraction waves is not apparent. Obviously this proposed explanation of flagellar motion is based on meagre information and requires further study, and conflicts with the views of Brown (1945).

As stated before, these studies provide little or no information concerning the nucleus, the blepharoplast or the reproductive forms of these organisms. Trypanosomes apparently in division have been frequently observed. The body is much larger than normally and a small flagellum may be seen coming out of it (Pl. V, fig. 17). It looks almost as though the new flagellum was being discharged from the body of the parent through a pore. In other cases where the reproduction has proceeded further there are two distinct flagellae and one enlarged body which, still further, begins to split at the posterior end. However, until observations can be made of internal events involving the nucleus and blepharoplast the sequences in division will not be clear.

## SUMMARY

*Trypanosoma cruzi* has been examined with the electron microscope. Crithidia and metacyclic forms of a blood agar culture have been studied and compared with the adult tissue forms, obtained in tissue cultures, from the same strain.

In all the forms studied, the internal structure of the body is not revealed because it is too thick for electron penetration. In places where a good penetration has been obtained the cytoplasm shows a fine striation in spiral or parallel arrangement. This striation can be digested with trypsin or by prolonged osmic acid fixation.

Dense spherical bodies of variable size can also be observed in the body portion. They can be dissolved with xylene.

The whole body of the trypanosome is covered by a fine, transparent sheath.

The flagellum is formed by the axoneme which consists of five or nine fibres. It can be followed throughout the whole length of the metacyclic and of the adult tissue forms. In the crithidia forms of the blood-agar culture, it disappears in the anterior part of the body. The whole flagellum is covered by a fine sheath. The axial fibre bundle can be digested with trypsin or by prolonged osmic-acid fixation. Both, the striation and the fibre bundle are contractile structures which could explain the movements of the trypanosome.

Some dividing forms are also described.

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## EXPLANATION OF PLATES

(Scales in all figures represent 1 $\mu$)

### PLATE I. *Trypanosoma cruzi*

Fig. 1. Crithidia form from blood-agar culture. Photograph of living trypanosomes with phase-contrast microscope.

Fig. 2. Crithidia form from blood-agar culture. Fixed with osmic acid for 10 min. Electron micrograph, × 7200. Taken with Philips Electron Microscope. Fine striations of cytoplasm are apparent in transparent part of body. Centre of body contains deeply stained granules and is too thick for electron penetration. Striated portion of body extends far down along proximal part of flagellum. Axial fibre bundle runs from tip of flagellum into body of trypanosome. The whole flagellum is covered by a thin sheath.

Fig. 3. Adult form from tissue culture shortly after extrusion from tissue cell. Electron micrograph, × 11,340. Considerable detritus from tissue culture is attached to trypanosome and surrounds it in the field of micrograph. The whole trypanosome is covered by a thin sheath. The inner structure of body portion is not revealed. Transparent part shows fine striations and deeply stained granules. The axoneme or axial fibre bundle can be followed throughout the whole length of trypanosome.

### PLATE II. *Trypanosoma cruzi*

Fig. 4. Crithidia form from blood-agar culture. Fixed with osmic acid for 10 min. Electron micrograph, × 11,200. Fine fibrillar striations of cytoplasm are clearly apparent and seem to have spiral arrangement. Fibres increase in number as one passes from tip to larger part of the body.

Fig. 5. Crithidia form from blood-agar culture. Fixed with osmic acid for 24 hr. Electron micrograph, × 7350. The fibrillar structure prominent in the preceding micrograph has been destroyed by prolonged osmium fixation. The membrane remains intact. *l.g.*, lipid granules.

Fig. 6. Same as fig. 4 above. Higher magnification, × 16,800. Micrograph shows fibres to have cross banded structure at *s*, and elsewhere, *m*, membrane.

Fig. 7. Crithidia form from blood-agar culture. Fixed with osmic acid for 10 min. and shadowed with chromium. Electron micrograph, × 18,200. Micrograph shows posterior tip of body with longitudinal striations of the cytoplasm in prominence, and spherical lipid granules (*l.g.*).

Fig. 8. Crithidia form from blood-agar culture. Fixed in osmic acid for 10 min. Electron micrograph, × 17,840. Micrograph shows especially well the origin of subthecal fibrils and their fine structure.

### PLATE III. *Trypanosoma cruzi*

Fig. 9. Crithidia form from blood-agar culture. Fixed and shadowed as trypanosome in fig. 7. Electron micrograph, × 9000. Micrograph shows proximal part of flagellum with fibrillar cytoplasm of body portion of cell, plus several lipid granules of varying sizes (*l.g.*).

Fig. 10. Crithidia form from blood-agar culture. Fixed with osmic acid for 3 min. and treated with xylene for 30 min. Shadowed with chromium. Electron micrograph, × 9000. Proximal part of flagellum, lipid granules *l.g.* partly removed by xylene.

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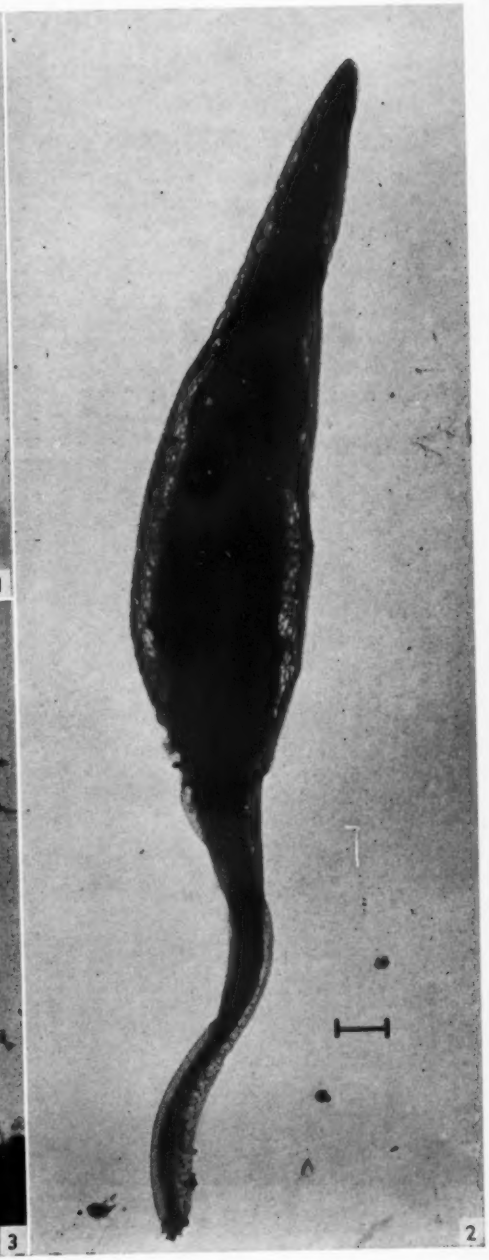
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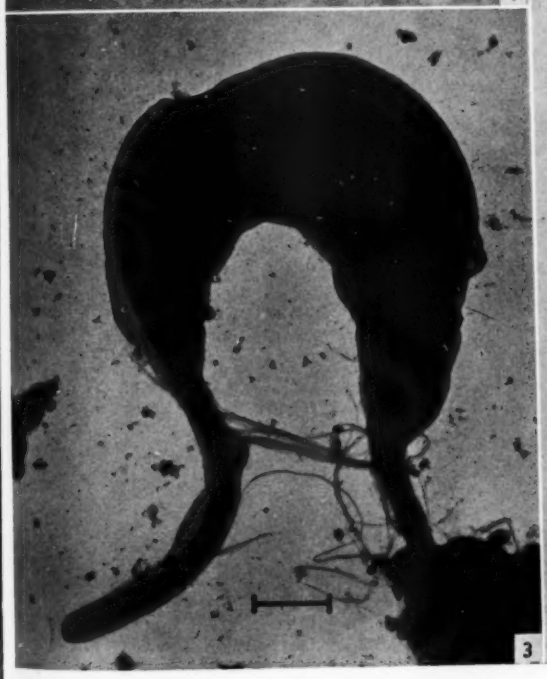
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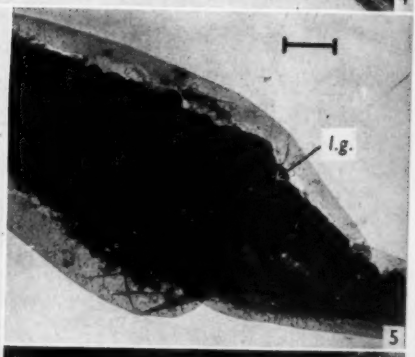
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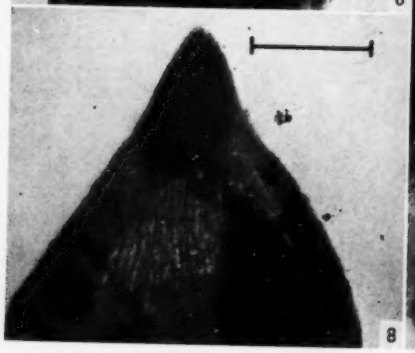
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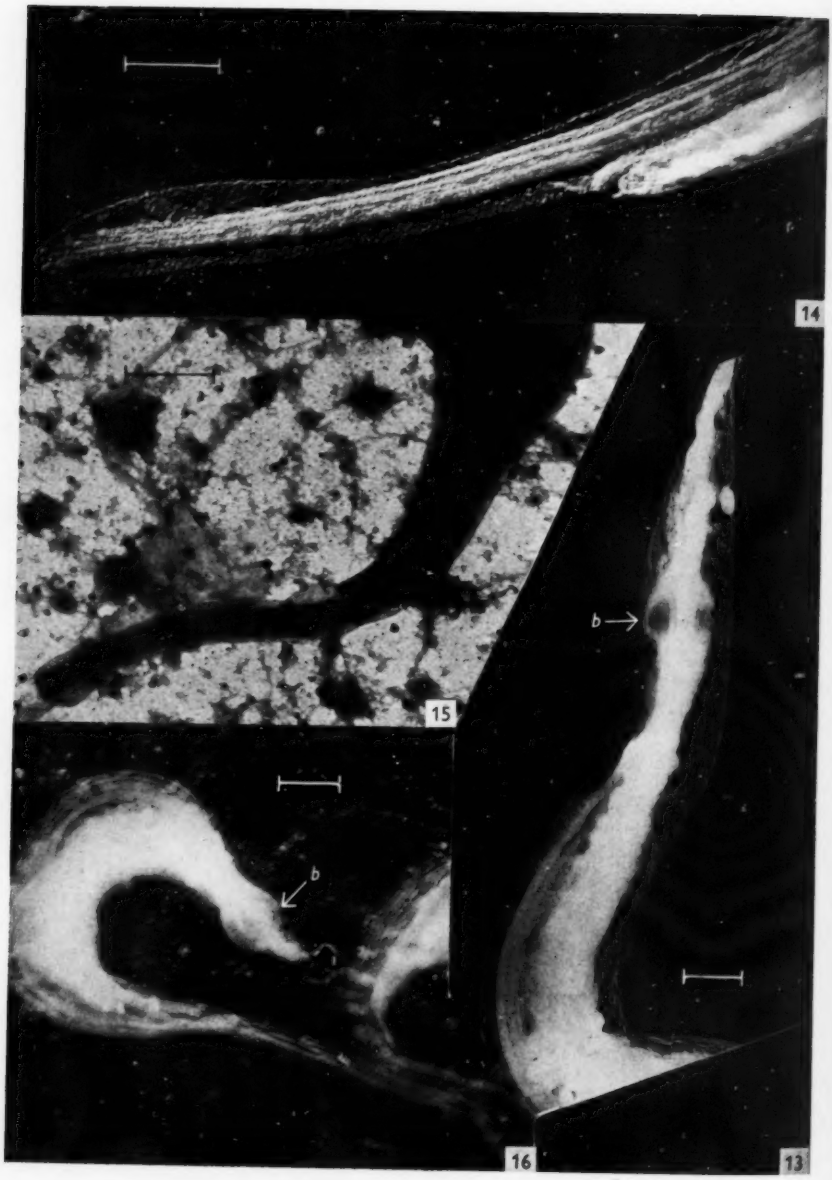
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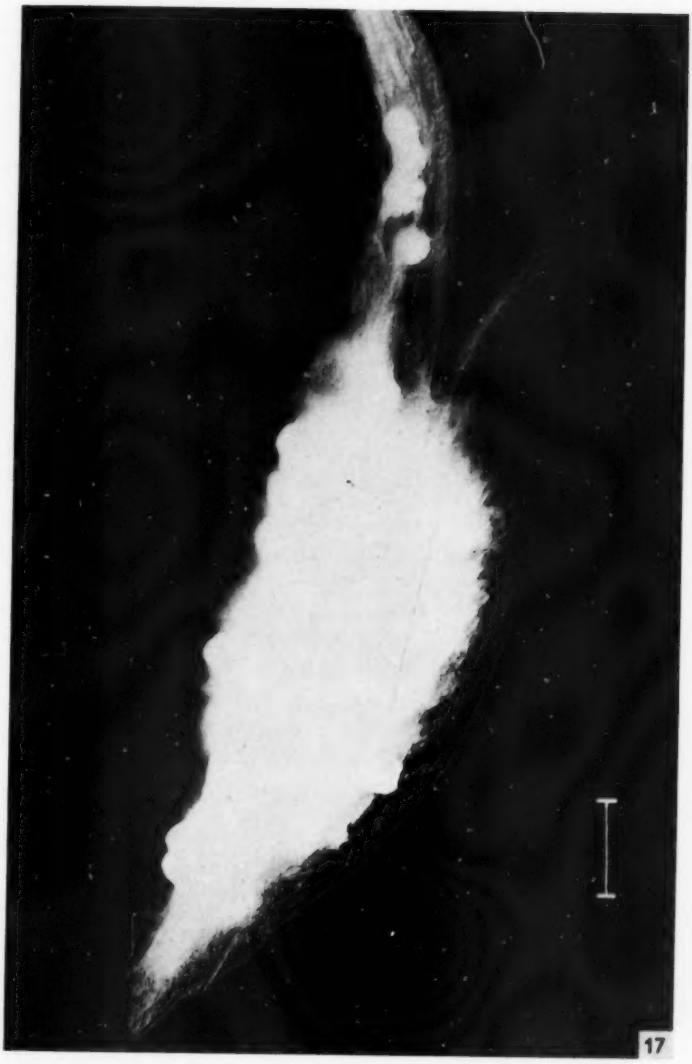
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Fig. 11. Crithidia form from blood-agar culture. Fixed with osmic acid for 10 min. Shadowed with chromium. Electron micrograph, × 9000. Axial fibre bundle apparently separated from striated layer in proximal part of flagellum; lipid granules *l.g.*

Fig. 12. Crithidia form from blood-agar culture. Fixed with osmic acid for 24 hr. Shadowed with chromium. Electron micrograph, × 9000. The prolonged fixation has destroyed most of the striated cytoplasm and the axial fibre of the flagellum. The remaining sheaths show distinct separation.

PLATE IV. *Trypanosoma cruzi*

Fig. 13. Metacyclic form from blood-agar culture. Fixed with osmic acid for 10 min. Shadowed with chromium. Electron micrograph, × 9000. Axoneme disappears shortly before blepharoplast in posterior part of the body. Blepharoplast (*b*) shows no structure but is traversed by some fibres.

Fig. 14. Distal part of flagellum from blood-agar form. Fixed with osmic acid for 10 min. Shadowed with chromium. Electron micrograph, × 14,400. Axial fibre bundle very distinct.

Fig. 15. Flagellum of adult form from tissue culture. Fixed with osmic acid vapours. Electron micrograph, × 13,400. Fibrillar structure of flagellum appears quite distinctly.

Fig. 16. Adult form from tissue culture. Fixed with osmic acid vapours. Shadowed with chromium. Electron micrograph, × 9000. Fibrillar structure of axoneme distinct. Body too thick for good electron penetration, shows some granules in transparent part. In blepharoplast (*b*) three fibres.

PLATE V. *Trypanosoma cruzi*

Fig. 17. Crithidia form from blood-agar culture. Fixed with osmic acid for 10 min. Shadowed with chromium. Electron micrograph, × 15,000. Large form (in division) sending out new flagellum. Body too thick for electron penetration.

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# *PSEUDOTRICHONYMPHA SERTANEJA* N.SP. (PROTOZOA, MASTIGOPHORA), FROM THE INTESTINE OF A NEW TERMITE (*RUGITERMES* SP.) COLLECTED IN BRAZIL

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(With 3 Figures in the Text)

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## INTRODUCTION

The flagellate protozoa of termites provide very interesting studies. Having collected an apprently new termite at the Experimental Farm of Sertãosinha, São Paulo State, Brazil, I sent some specimens to Prof. A. Emerson, the well-known termitologist at the University of Chicago, who identified them as soldiers and nymphs of a new species of the genus *Rugitermes*.

In this termite was found an interesting species of *Pseudotrichonympha*, the study of which has involved detailed examination of both the generic and specific characters of this genus, as well as a description of the new species.

## *PSEUDOTRICHONYMPHA:* GENERIC AND SPECIFIC CHARACTERS. DISCUSSION

From a study of the literature on trichonymphids of the genus *Pseudotrichonympha*, and from my own observations on many species found by me in Indian termites, and, recently, in *Heterotermes tenuis* Hagen from Brazil, the characteristics of the genus can be described in a systematic and comprehensible way, with due consideration of characters which are important for the differentiation of species.

Following the nomenclature that I have already employed, and without attempting to homologize the terms with their homonyms in higher Metazoa, the body of *Pseudotrichonympha* can be divided into three segments, shown schematically in Fig. 1.

The first segment or head (the cap of Porter, Koidzumi; operculum of Kofoid and Swezy) (*a*) which has the form of an umbrella or the cap of a mushroom, is a generic character; it is usually hyaline but sometimes with a fine, alveolated, lace-like structure, which may be either a specific or generic character, and is seen only in well-stained preparations. The base of the head is reinforced by two strongly siderophilé threads (*c*), which I term siderophile barbellae, one on each side, with

their tips floating freely a little beyond the angle of confluence of the anterior and posterior margins of the mushroom-like cap. This is a generic character.

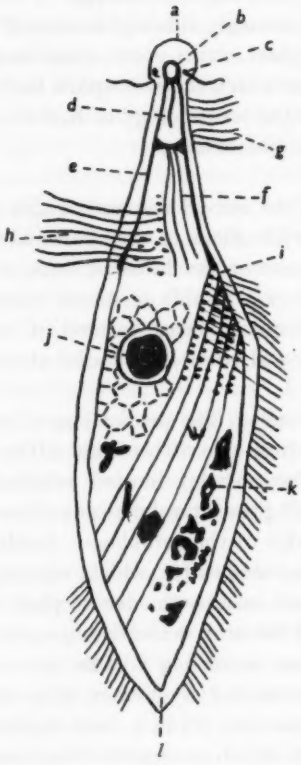


Fig. 1

Fig. 2

Fig. 1. Schematic representation of a *Pseudotrichonympha*. *a*, head; *b*, blepharoplast; *c*, basal granules with siderophile barbellae; *d*, centroblepharoplast with axoneme and lower (inferior) ring; *e*, triangular area of the anterior part of the body, with strong borders; *f*, myonemes; *g*, flagella of the neck; *h*, long, mobile flagella of the second series; *i*, leiotrophic striae and flagella of the third series; *j*, nucleus in alveolar zone; *k*, wood and inclusions; *l*, posterior pole of the body devoid of flagella.

Fig. 2. General morphogical aspects of *Pseudotrichonympha sertaneja*. Camera lucida outlines.

The second segment or neck (the nipple of Porter, Koidzumi; rostrum, rostral tube of Duboscq and Grassi, Kirby; centroblepharoplast of Kofoid and Swezy) consists of:

(*a*) A central, strongly siderophile tube, which, at its upper end, supports a more or less lenticular, hemispherical or mamillated basal granule, according to the species, representing the true blepharoplast (*b*).

(*b*) An axial filament in the middle of the siderophile tube (*d*). This generally fades out distally.

(*c*) A more or less circular ring in the basal portion of the neck, from which the myonemes (*f*) of the upper portion of the third segment emerge.

This central tube, whose very thick and strongly siderophile lateral borders (*e*) take their origin at each side of the blepharoplast in two, more or less basal granules, either independently or united to those from which the siderophile barbellae arise, is named the centroblepharoplast (*d*), after the terminology of Kofoid and Swezy, as it plays an important role in the mitotic phenomena.

(*d*) A surrounding layer of ectoplasm.

These are the basic generic characters of the second segment, which in different species may show such strictly specific modifications of their form as the central tube being hourglass-like, cylindrical or a more or less flattened cone, or may have superadded organelles such as a meniscus or a double meniscus reinforcing the blepharoplast or the inferior ring, the ectoplasm being formed of one or more layers, a campanula surrounding the neck, and bottle-like bodies attached to the lateral upper basal granules.

The third segment or body, properly so called. The proportions of the anterior to the posterior part of the segment vary, from one-fifth to four-fifths.

The anterior or upper part is a triangular area (*e*), limited externally by two strongly siderophile diverging lines, continuing the lateral margins of the centroblepharoplast to their intersection with the edge of the ectoplasm. In this zone the following organelles are situated: (1) the myonemes (*f*), which, starting from the lower circular ring of the centroblepharoplast, occupy the deeper plane of the area and give very great mobility to this part of the area, which is a generic character. These myonemes may take various forms, according to the species, such as appearing as single threads, branched dichotomized myonemes, whip-like bundles, all of which represent strictly specific characters; (2) in a more superficial plane there are the basal granules and rodlets from which emerge the long mobile flagella of the second series (*h*).

The posterior part of the body has a rather uniform structure. It is irregularly alveolated, surrounded by a single or double layer of ectoplasm, the whole surface crossed by longitudinal leiotropic striae (*i*). The endoplasm has numerous inclusions (wood particles, parasites, etc.) (*k*). The nucleus (*j*) lies in this zone. Its position varies not only according to the species but even in different individuals of the same species. The nucleus is spherical, composed of a densely packed endosome (karyosome), with chromatin granules in various dispositions according to the stage of evolution of the parasite. A clear halo separates the endosome from the nuclear membrane, whose thickness and siderophilia vary in different species, constituting specific differences. Three series of flagella, which provide generic characters, are present on the body of the parasite.

Series 1. Short, immobile flagella, surrounding the ectoplasm of the neck (*g*).

Series 2. Long, very mobile flagella, emerging from the basal granules of the anterior triangular area of the body (*h*).

Series 3. Semi-mobile flagella, emerging from the basal granules embedded in the leiotrophic striae which cross the ectoplasm of the body (*i*).

The cap or head has no flagella. They are also lacking on a small area at the posterior end of the body (*l*).

Specific characters of the flagella are represented by their size, uniformity in each series or gradual increase in the lower parts, as happens especially in the third series, and the extent to which the body of the organism is covered by flagella.

The characteristics described in the preceding paragraphs are very similar to those found in the genus *Trichonympha*. Until 1942 I considered (de Millo, 1927, 1929, 1939) that the main differences between *Trichonympha* and *Pseudotrichonympha* were the presence in *Trichonympha* of two important organelles, the rostral articulation (citartrosi of Grassi) and the parabasal body. However, I have seen definite evidence of a parabasal body in at least two species of *Pseudotrichonympha*, one from *Coptotermes heimi* of India, in fine preparations made by an Indian research worker, Mr Vittal, whose work is not yet published, and the second from the intestine of *Heterotermes tenuis* Hagen, in a paper of mine in the press, to which the name *Pseudotrichonympha paulistana* n.sp. had been given. In the parasite from *Coptotermes heimi*, the parabasal was in the form of a bundle of threads coming down to one side of the nucleus and touching it underneath. In *Pseudotrichonympha paulistana*, it is a more compact faggot, often divided into two branches distally. Thus, it seems that the parabasal body may also exist in the genus *Pseudotrichonympha*.

If we consider that Grassi (1917) himself stated that the citartrosi are seen in some species but cannot be seen in others—to quote his own words, 'suppongo che exista in tutti le forme, ma molto poco sviluppata', there is ground for asking what is the taxonomic position of *Pseudotrichonympha vis-à-vis Trichonympha*. Personally, I have not seen citartrosi in any of the specimens studied during my researches, but in some of Mr Vittal's preparations, a faint indication of a rostral split was sometimes observed. For the present, no definite statement can be given and further study is needed.

### **Pseudotrichonympha sertaneja** n.sp. (Figs. 2, 3)

The morphology of *Pseudotrichonympha sertaneja* n.sp. is as follows:

The general body form (Figs. 2, 3) is narrow, elongate, more or less cucumber-like with pointed ends.

*The head.* Hyaline, easily collapsing, its base reinforced by siderophile barbellae, whose basal granules are hardly visible.

*The neck.* Centroblepharoplast conspicuous, strongly siderophile, with a circular blepharoplast bordering a central cavity. Axoneme not visible. Lateral margins of the centroblepharoplast diverging. Inferior ring bounded by a double contour. The basal part of the centroblepharoplast is larger than the anterior one, so that its outline is cone-shaped. Ectoplasm constituted by a single fine, thin membrane.

*The body.* Anterior portion limited by two thick, strongly siderophile lines continuing the lateral borders of the centroblepharoplast, fading posteriorly after their intersection with the ectoplasm. No citartrosi or rostral articulation.

Myonemes discontinuous, formed by rodlet spassing posteriorly in linear diverging directions. Basal granules of the second series of flagella are not spread over all this zone but are concentrated in its basal part in one or two bands about 5–6$\mu$ in width.

The remainder of the body is irregularly alveolated and is crossed by parallel oblique striae which are strongly leiotrophic. The endoplasm contains inclusions and wood particles throughout the body. The outline of the ectoplasm is undifferentiated throughout its extent.

The nucleus is spherical, situated roughly in the anterior third of the body. The endosome (karyosome) has a marked membrane with packed chromatin masses within, surrounded by a delicate halo 1–1·5$\mu$ wide. No mitotic figures were seen.

*Flagella.* First series: short, immobile flagella not confined to the neck but covering the entire ectoplasm up to the intersection of the siderophile lines of the first body portion with the ectoplasm. Second series: long, mobile flagella, not proceeding from the whole anterior zone of the body but restricted to its base, where the basal granules from which they arise are situated. Third series: very short flagella of uniform size, not gradually increasing in length as in many Pseudotrichonymphidae. They originate in minute basal granules in the leiotrophic striae and are absent from the posterior 3–5$\mu$ of the body length.

*Measurements.* Length/width, 250/45, 260/60, 300/60, 320/50$\mu$. Nucleus 18–20$\mu$ diameter. Flagella: series 1, 6–8$\mu$; series 2, 15–18$\mu$; series 3, 2–3$\mu$. Centroblepharoplast, 5–6$\mu$. Distance between striae, 2$\mu$.

*Specific characters.* (*a*) Myonemes discontinuous (the first time this feature has been seen by me); (*b*) striae not longitudinal but oblique and decidedly leiotrophic; (*c*) flagella of first series not confined to the neck but continuing to the anterior (upper) part of the body; (*d*) flagella of the second series only on the basal part of the triangular anterior area of the body; (*e*) flagella of the third series very short and of uniform size; (*f*) ectoplasm very thin, formed of one layer only.

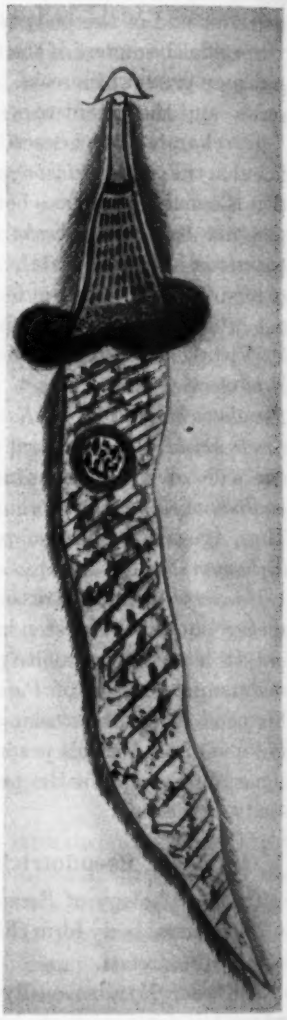

Fig. 3. *Pseudotrichonympha sertaneja* n.sp. Detailed morphology.

From consideration of the foregoing characters, the species is a new one, which I name *Pseudotrichonympha sertaneja* n.sp., the specific name being derived from the locality where the new species of *Rugitermes* was collected.

SUMMARY

This paper contains a discussion of the generic and specific characters of *Trichonympha* and *Pseudotrichonympha*, with a detailed account of the morphology of *P. sertaneja* n.sp. from a new species of *Rugitermes*.

The basic generic and specific characters of each organelle of *Pseudotrichonympha* are considered in detail. The genus *Pseudotrichonympha* has been separated from *Trichonympha* by the absence of the rostral articulation (citartrosi) and parabasal body. But in at least two species of *Pseudotrichonympha* a parabasal body has been found, and as Grassi himself believed that citartrosi, however feebly developed, must exist in all *Pseudotrichonympha*, the taxonomic position of *Pseudotrichonympha needs further investigation.*

The morphology of *P. sertaneja* n.sp. has the basic generic characters. The specific characters are discontinuous myonemes, oblique instead of longitudinal leiotropic striae, flagella of the first series covering not only the neck but the upper portion of the body, flagella of the second series confined to the basal part of the anterior triangular area of the body, flagella of the third series very short and of uniform size and simple outline of the ectoplasm.

The flagellate is regarded as a new species, named *P. sertaneja* n.sp. The host is a new species of *Rugitermes* from São Paulo, Brazil.

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# ON A NEW SPECIES OF *STEPHANONYMPHA* (PROTOZOA, MASTIGOPHORA) FROM THE INTESTINE OF THE BRAZILIAN TERMITE, *NEOTERMES HIRTELLUS*

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(With Plate VI)

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## INTRODUCTION. HISTORICAL

The genus *Stephanonympha* was created by Janicki (1915) for the species *S. silvestrii*, parasite of an Hawaiian termite, which, wrongly classified as *Calotermes castaneus* Burmeister, was later on correctly identified as *Neotermes connexus* Snyder. This flagellate is oval, metabolic, possesses numerous fusiform nuclei located in its anterior part and disposed in concentric circles or spiral bands. Each nucleus shows on its anterior pole one axostylar filament, one parabasal body and four flagella. The axostylar filaments coming downwards form a longitudinal bundle which ends at a short distance from the posterior pole. Two forms were described: *minor* and *major*, 69 and 136$\mu$ long respectively, the former with about 100 nuclei disposed in two to four, generally three ranks, the latter with nearly 150 nuclei (Janicki, 1915).

Grassi (1917) described two new varieties, *Stephanonympha silvestrii* var. *cryptotermitis havilandi*, 60$\mu$ long, with a small lenticular parabasal, axostylar filament attached to the blepharoplast and two to four flagella; and *S. silvestrii* var. *neotermitis erythrei*, which has the peculiarity of possessing a plasmatic mass instead of a definite parabasal body. These quadrinomial designations have been changed by Kirby to *S. havilandi* and *S. erythrei*.

Kirby (1926) described *Stephanonympha nelumbium*, a parasite of *Cryptotermes hermsi*. It was spherical or ovoid, often with the posterior pole pointed, axostylar bundle protruding as a truncated cone, the anterior region of the parasite clearer in contrast with the remainder of the body, body 27–69$\mu$ (average 45$\mu$) long, 19–40$\mu$ (average 27$\mu$) wide. Nucleus oval, 1–2$\mu$, sometimes with a herniated granule on the posterior pole. There is a large blepharoplast to which is attached a conical eminence giving rise to one of the flagella, whilst the other three emerge as a bundle placed laterally. Parabasal body spherical, smaller than half the nucleus and united to the blepharoplast by a fibril. Spirosymbionts forming a tuft on the posterior pole are frequently present.

In 1928 Bernstein described her *Stephanonympha dogieli*, a parasite of *Hodotermes murgabicus*. It was pyriform, posterior pole pointed, 30–160 $\mu$ (average 80 $\mu$) long, with four to twenty-four nuclei at the anterior pole, though often located at different levels of the body. Each mastigont possessed one nucleus, one axostyle, one blepharoplast (named centrosome by the author), one basal granule, one parabasal body and four flagella arising from the basal granule which is united to the blepharoplast by a fibril.

Froilano de Mello (1946) described *Stephanonympha reenstiernai* from an Indian *Cryptotermes*. It was generally ovoid, with the posterior pole pointed, often cucumber-shaped. Numerous spherical forms, either large or small, from 22–32 to 52–55 $\mu$ long; 20–22 to 48–50 $\mu$ wide. Nucleus ovoid, with posterior pole pointed. Number of nuclei from 44–55 to 80–100, often uncountable, disposed in 3–5–9 rows. The interesting peculiarity of this parasite is that in the small circular forms the whole body is full of nuclei. The blepharoplast is large, with two fibrils starting from it, one a large one attached to the parabasal which covers the nucleus as a cap or remains at its side, the other attached to a small basal granule from which emerge the axostyle and four flagella, 28–30 $\mu$ long. Axostylar bundle not protruding. Endoplasm divided into three zones: external, alveolar, containing wood and inclusions; median, axostylar; and intermediate, clearer, distinctly separated from the external zone.

In the structure of the Calonymphidae, to which the genus *Stephanonympha* belongs, some organelles have been undetected by the authors, because they are located in such a small area occupied by and crowded with mastigonts: for example, the cresta, similar to the cresta of the Devescovinids, which has been described for the first time in a *Metastephanonympha*, parasitic in an Indian millipede (de Mello & Uttangi, 1950). This organelle I have constantly found in the *Stephanonympha* of *Cryptotermes havilandi*, imported and established in Brazil (Santos), and also in another parasite of the same genus harboured by a new species of *Rugitermes*, collected at the town of Sertãosinha. In consequence, I believe that the cresta is an essential organelle of the Calonymphidae, at least in the genera *Stephanonympha* and *Metastephanonympha*.

### **Stephanonympha campinae** n.sp. (Pl. VI)

The termite host of this new *Stephanonympha* has been kindly identified as *Neotermes hirtellus* (Silvestri) by Prof. A. Emerson of the University of Chicago, to whom our best thanks are due. Although numerous species of *Neotermes* have been studied in connexion with their intestinal Protozoa, it is the first time that the Entozoa of *N. hirtellus* have been recorded. The morphology of this flagellate is as follows:

*Body.* Broad oval, with the posterior pole somewhat less round than the anterior one (Pl. VI, fig. 1), or sometimes slightly pointed, giving to the parasite a pyriform appearance with a narrower posterior end (Pl. VI, fig. 3). As the parasite is metabolic, some irregular forms are found, the irregularity often being due to the action of the fixatives. No regularly spherical forms were seen.

*Nuclei.* Ovoid, with well-defined membrane and the endosome (karyosome)

surrounded by a very small, clear space separating it definitely from the membrane (Pl. VI, fig. 4B). The chromatin is disposed in packed masses or granules, centrally located and not herniating.

*Structure of the mastigont* (Pl. VI, fig. 4A). At the anterior pole of the nucleus there is a rather large blepharoplast from which the following organelles emerge: (*a*) a tiny fibril connecting the blepharoplast to a minute basal granule to which the parabasal border is attached; (*b*) a cresta, bacilliform, strongly siderophile; (*c*) four long, slender flagella; (*d*) the axostylar filament which, bordering more or less closely the nuclear membrane, runs downwards to form the axostylar bundle.

*Parabasal body.* Oval, as large as the nucleus, less siderophile than the chromatin or the cresta, and uniformly stained. It is closely attached to the nucleus.

*Position of the nuclei* (Pl. VI, figs. 1, 3). Usually at the anterior pole, where they form a bunch of closely packed masses. Rarely, however, some of them are spread at other levels of the body (Pl. VI, fig. 2).

*Number of nuclei and nuclear rows.* The following figures have been recorded:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Number of nuclei | 30 | 22 | 15 | 30 | 25 | 40 | 60 |
| Number of rows | 5 | 3 | 3 | 4 | 4 | 5 | 6 |

Hence, fifteen to sixty nuclei and three to six rows may be present.

*Axostylar bundle.* This occupies the median area of the body, passing posteriorly as a multi-fibred structure which, though it sometimes ends some distance from the inferior boundary of the ectoplasm (Pl. VI, fig. 1), in most cases protrudes beyond the body as a pointed, arrow-like protuberance, more or less uniformly stained in its posterior portion (Pl. VI, figs. 2, 3).

*Ectoplasm.* A thin but definite line not giving insertion to any symbionts.

*Endoplasm.* Alveolar, containing wood particles and inclusions.

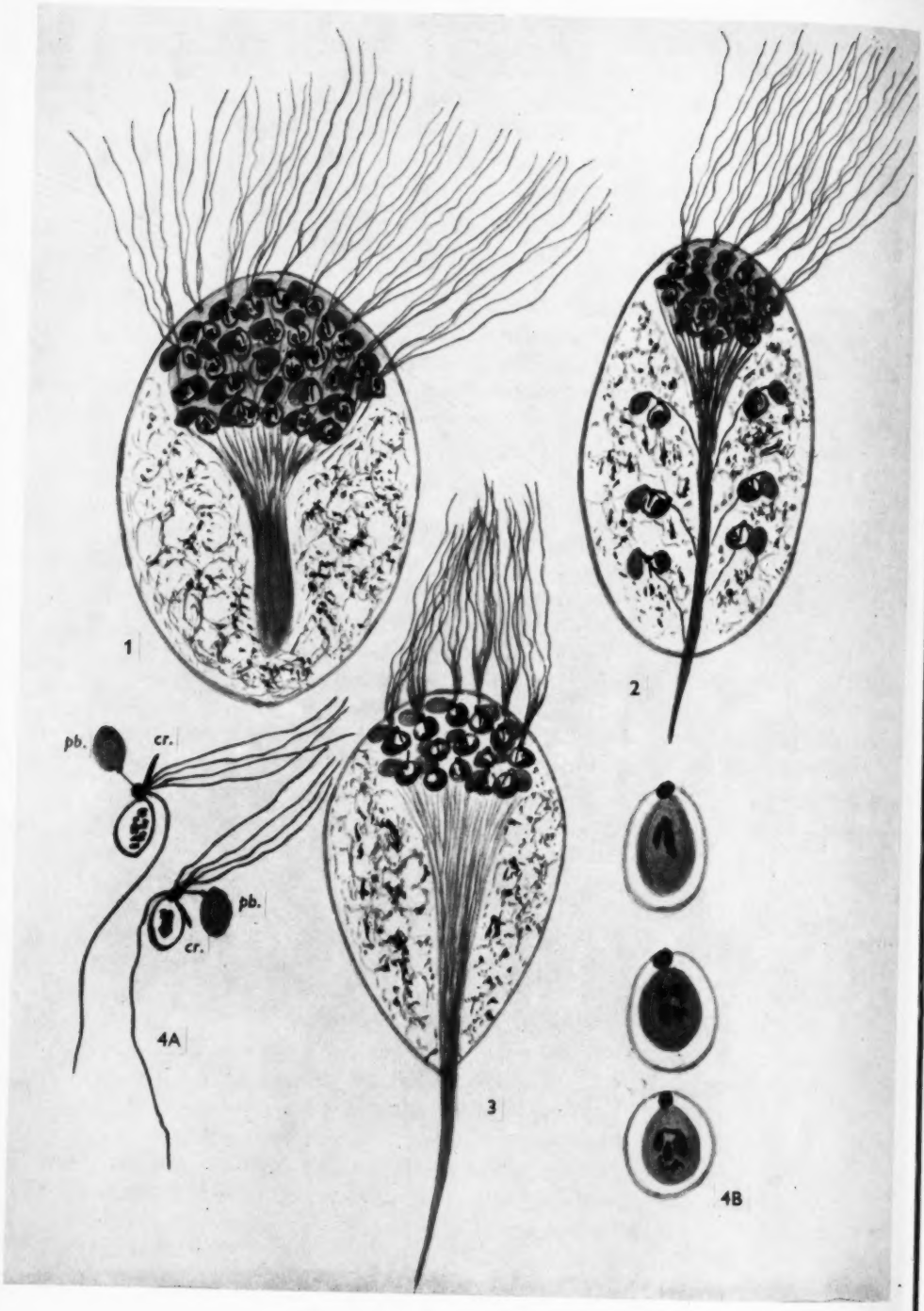
*Distinctive specific characters.* The two main ones are (*a*) the large parabasal body; (*b*) the very long flagella. Secondary specific characters—absence of circular forms and abundance of organisms in which the axostyle protrudes beyond the ectoplasm.

*Measurements in microns.* Length/width 80/50, 70/40, 100/90, 60/45, 100/50, 60/30. Nucleus, 2·0–2·5; parabasal, as large as the nucleus; flagella, 35–40; protruding part of the axostyle, up to 20$\mu$.

## SYSTEMATIC POSITION

The number of nuclei in this new species of *Stephanonympha* is much smaller than in *S. silvestrii* and its axostylar bundle protrudes. *S. havilandi* has a very small parabasal. *S. erythrei* has a plasmatic mass instead of a parabasal. *S. nelumbium* has a parabasal less than half the nucleus in size and possesses a conical eminence attached to the blepharoplast. *S. dogieli* has a smaller number of nuclei, located at different levels. *S. reenstiernai* is a small species with numerous nuclei.

It is considered, therefore, that the flagellate from *Neotermes hirtellus* is a new species, which is named *Stephanonympha campinae* n.sp., the species name being associated with that of the Brazilian town where the host termite was collected.




*Stephanonympha campinae* n.sp.

SUMMARY

An illustrated account of a new species of *Stephanonympha* from the Brazilian termite, *Neotermes hirtellus* Silvestri, is given.

An historical introduction provides concise accounts of the previously described species of *Stephanonympha*.

The morphology of the new *Stephanonympha* is described in detail and illustrated.

Differential characters from previously described species of *Stephanonympha* are noted, the conclusion being that the *Stephanonympha* of *Neotermes hirtellus* is a new species from a new host, to which the name *Stephanonympha campinae* n.sp. is given.

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EXPLANATION OF PLATE VI

Figs. 1, 2. *Stephanonympha campinae* n.sp. Oval forms.

Fig. 3. *Stephanonympha campinae* n.sp. Pyriform type, with protruding axostylar bundle.

Fig. 4A. Structure of a mastigont, showing cresta (*cr.*) and parabasal (*pb.*).

Fig. 4B. Nucleus showing chromatin granules in the endosome (karyosome).

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# A MORPHOLOGICAL STUDY OF A FISH TAPEWORM, *PROTEOCEPHALUS POLLANICOLA*

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(With 7 Figures in the Text)

## INTRODUCTION

*Proteocephalus pollanicola*, parasitic in the alimentary canal of Lough Neagh pollan, *Coregonus pollan* Thompson, was first recorded by Gresson (1952). The present account is based upon the examination of specimens obtained during the spring and summer of 1950, 1951 and 1952, and the spring and early summer of 1953.

In the preliminary note cited above Gresson pointed out that examination of the intestine of perch, *Perca fluviatilis* L., captured in Lough Neagh and the Lower Bann River in close proximity to the lough, and of trout, *Salmo trutta* L., and pike, *Esox lucius* L., obtained from some of the rivers flowing into the lough, failed to reveal the presence of proteocephalids. Since the publication of this note the writers have examined the alimentary canals of several perch and of some large trout caught in Lough Neagh. In the spring of 1953 the contents of the intestine of fifty eels, *Anguilla anguilla* L., from Lough Neagh were carefully investigated. These fish were found to be devoid of specimens of *Proteocephalus*. It may be concluded, therefore, with a reasonable degree of certainty, that *P. pollanicola* is confined, in Lough Neagh at least, to the alimentary canal of *Coregonus pollan*. It is worth mentioning that during the past four years, in addition to the investigations recorded above, research students in the Department of Zoology of the Queen's University have examined the intestial faunna of the following local fresh-water fishes: trout, *Salmo trutta* L.; salmon, *S. salar* L.; pike, *Esox lucius* L.; perch, *Perca fluviatilis* L.; bream, *Abramis brama* L.; rudd, *Scardinius erythrophthalmus* L.; and of a small number of minnow, *Phoxinus phoxinus* L., and three-spined sticklebacks, *Gasterosteus aculeatus* L. Members of the genus *Proteocephalus* were not found in any of the fish examined.

La Rue (1914) listed the proteocephalids which at that time were known as parasites of the genus *Coregonus* and of other fish. According to his account *Proteocephalus filicollis* was the only member of its genus identified from Ireland. *P. filicollis* was first recorded by O'Bryen Bellingham (1844), under the name of *Taenia filicollis*, as present in Ireland in the intestines of three-spined sticklebacks, *Gasterosteus aculeatus*. He stated that he found several specimens, but did not mention the part of the country from which the sticklebacks were obtained. It would appear that the only other member of the genus *Proteocephalus* recorded from Irish fresh-water fish is *P. pollanicola* (Gresson, 1952).

Most of the descriptions of that part of the female reproductive system of

proteocephalids which lies posterior to the isthmus of the ovary are incomplete. As the writers possess favourable preparations, they consider that a detailed description of the reproductive system would add to our knowledge of the group. In many cases previous workers seem to have been content to follow the vagina or seminal canal as far as the isthmus of the ovary and to ignore the various ducts that are situated between the latter and the posterior boundary of the proglottis, or else to figure a coiled tube lying between the lobes of the ovary. In either case there is little or no attempt to define the relationship between the vagina, oviduct, yolk ducts and uterus. Some few authors, however, have given more detailed accounts or figures of the reproductive apparatus of proteocephalids, and some of these will be discussed in the light of the present observations.

## MATERIAL AND METHODS

The alimentary canal of *Coregonus pollan* was removed and placed in a glass dish containing physiological saline. The canal was then opened longitudinally and shaken vigorously. When the mucus content of the gut was great, tap water, heated to 25–30° C., was used instead of saline, because the mucus was more soluble in tepid water. In most cases the canal was removed within 24 hr. after the fish were captured by local fishermen, but occasionally the pollan had to be kept in a refrigerator for a further period of about 20–24 hr. It was found advantageous to leave the intestines in physiological saline or else in tap water for an hour or more. The cestodes then tended to separate out from the intestinal contents and to be comparatively free of mucus. The scolices were often firmly attached to the pyloric caeca, but frequently entire worms were found free in the lumen of the small intestine. The number of worms lying free in the intestine seemed to increase after the fish had been placed in a refrigerator prior to dissection, or when the alimentary canal was kept for several hours in saline. Usually the worms exhibited active signs of life even after long periods in saline or after the fish had been kept in the refrigerator for more than 24 hr. Many of the larger worms were observed to discharge ova.

Specimens were placed between two glass slides and fixed in 4% formalin or in 70% alcohol. They were subsequently stained in aceto-carmine or in Ehrlich's haematoxylin and mounted whole. Some specimens before fixation in formalin or alcohol, were placed in water which had been heated to 75° C. This treatment caused expansion of the proglottids.

Strobila were cut into short lengths and fixed in Bouin's picro-formol or in 4% formalin. Sections were subsequently cut at 8, 10, 15 and 20$\mu$ in thickness and stained in iron haematoxylin and light green or in aceto-carmine or Ehrlich's haematoxylin. Some of the sections stained with Ehrlich's haematoxylin were counterstained with light green. The thick sections were found to be particularly useful for following the course of the ducts of the reproductive system. In addition, specimens were observed under the microscope when they were alive. Examination of stained sections under a $\frac{1}{12}$ in. oil-immersion objective was found to be necessary for the determination of the minute structure of the parts of the reproductive system.

## OBSERVATIONS

### *General features*

Specimens of *Proteocephalus pollanicola* vary very considerably in length. Worms in which the reproductive system is fully mature are between about 2·5 and 30 cm. in total length. The width, taken at the widest region of the strobila, is more constant and varies between 1·25 and 2·5 mm. The greatest breadth of most of the specimens is 1·5–2·0 mm.

The scolex (Fig. 1) is provided with a well-marked apical, functional fifth sucker with an inside diameter of 0·1 mm. to about 0·15 mm., according to the degree of expansion or contraction of the organ. The four ordinary suckers are very muscular and in the living animal they exhibit considerable movement. They vary between about 0·1 and 0·25 mm. in breadth.

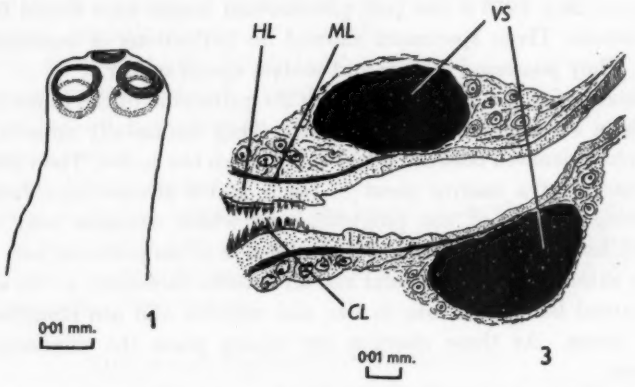
The 'neck' is approximately of the same diameter as, or slightly narrower than, the scolex. This region varies considerably in length. In some of the animals examined strobilization begins almost immediately behind the scolex, while in others the visible signs of strobilization are not apparent for 4 or 5 mm. or even for a greater length beyond the scolex.

The young segments situated towards the anterior end of the strobila are 4·5 times broader than they are long, while the mature proglottids are 2·5 times broader than long. In some of the worms the terminal proglottis was rounded, a fact which indicated that the strobila was complete. The genital atrium lies approximately at the middle of a proglottis and is irregularly alternating. The cuticle is in two parts, an outer comidial and an inner homogeneous layer (Hyman, 1951); underlying this is a basement membrane and subcuticular circular and longitudinal muscle fibres. Numerous subcuticular cells with elongate nuclei are present between these muscles and the parenchyma. The parenchymal muscle sheath, external to the reproductive organs, consists of bundles of longitudinal fibres and two bands of well-developed transverse fibres which constitute dorsal and ventral muscle sheaths.

The osmoregulatory system consists of two longitudinal canals on each side of the strobila, a wide transverse vessel posterior to the ovary connecting the two ventral canals, and numerous fine ducts originating from the dorsal and ventral longitudinal trunks and presumably terminating in flame cells. The longitudinal canals lie in the medullary parenchyma. The inner or ventral canal is situated lateral to the vitellaria. The outer or dorsal canal runs through the vitellaria and consequently tends to be obscured in the older proglottids. It follows a wavy course and has a narrower lumen than the ventral canal.

The majority of the ducts originating from both the dorsal and the ventral longitudinal trunks extend through the medullary parenchyma, where they form a branched system of fine tubes. Ducts were also observed in the cuticular region where it is possible that some terminate in 'foramina secundaria' (Wardle & McLeod, 1952). In stained specimens which possess a rounded terminal proglottis, a single wide duct or bladder with an opening to the exterior is often visible at the extreme posterior limit of the strobila. In some cases at least one longitudinal

vessel on each side was seen to communicate with this bladder. A plexus of osmoregulatory canals was identified in the scolices of some of the specimens examined; it was not possible, however, to follow the arrangement of the finer ducts.



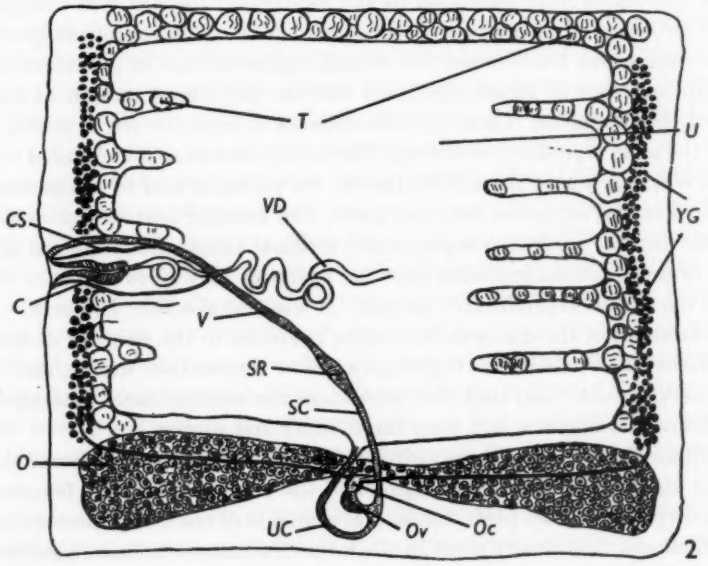




Fig. 1. Scolex.

Fig. 2. Diagrammatic drawing to show the principal features of the male and female reproductive systems.

Fig. 3. Part of the vagina in the vicinity of the genital atrium to show the lining and the vaginal sphincter.

*Key to figures:* *C*, cirrus; *CS*, cirrus-sac; *CL*, comidial layer; *EC*, external layer of cells; *HL*, homogeneous layer; *ML*, muscle layer; *O*, ovary; *Oc*, oocapt; *Ov*, oviduct; *SC*, seminal canal; *SR*, seminal receptacle; *T*, testes; *U*, uterus; *UC*, uterine canal; *V*, vagina; *VD*, vas deferens; *VS*, vaginal sphincter; *WO*, wall of ovary; *YC*, yolk cell; *YD*, yolk duct; *YG*, yolk glands.

The material was not satisfactory for the detailed examination of the nervous system, but ganglia were observed in whole mounts of scolices and sections of longitudinal cords and commissures were visible in the proglottids.

The vast majority of worms recovered during the investigation were mature. However, in May 1953 a few post-plerocercoid stages were found free in the intestinal mucus. These specimens showed no indications of segmentation nor of genitalia. They possessed a large and mobile apical sucker.

The distance from the scolex at which the primordia of the reproductive organs are visible in whole mounts varies slightly. They are usually apparent in the part of the strobila situated between 2 and 3 mm. from the scolex. The visible primordia at first consist of a narrow sheet of tissue which stains more darkly than the neighbouring regions of the proglottis and which contains very small deeply stained bodies. This area lies in the central region of the posterior part of a segment. It rapidly extends in both a lateral and an anterior direction; at the same time the deeply stained bodies increase in size and number and are identified as the developing testes. As these changes are taking place the vitellaria make their appearance.

When the testes have extended to the vicinity of the anterior margin of the segment two new structures are clearly visible. One is a narrow strip of deeply stained tissue which lies between the central region and one of the lateral margins. This strip increases in length and soon reaches the lateral margin at about the middle of the proglottis. Later, it differentiates to form the cirrus pouch and the ducts of the male reproductive system. The second structure is a round or oval mass of tissue which arises in the middle line at the posterior end of a segment. It increases in size and separates into two parts. The anterior part elongates and gives rise to the vagina, seminal receptacle and seminal canal. Examination of a large number of preparations indicates that the posterior part gives origin to the other ducts of the female reproductive system. It was not possible, however, to follow the development of the ducts in the region posterior to the isthmus of the ovary. It is probable that cells in this region give rise to the oviduct, uterus, and possibly to the vitelline ducts, and that at a later stage the seminal canal extends into the tissues below the isthmus and joins the oviduct and uterus.

The primordia of the ovary are visible at an early stage as two separate elements. When the tissue from which the vagina and the other ducts of the female system arise has divided into two parts, the lateral elements of the ovary increase in length and in thickness, and finally meet in the form of a narrow isthmus anterior to the oviduct. After this stage is reached no new structures are developed, and those already present, through the processes of growth and differentiation, rapidly assume their final form.

The following are features which the authors consider to be characteristic of *P. pollanicola*.

*Suckers* (four). Muscular. Inside diameter (fixed preparations) 0·1–0·25 mm.

*Apical sucker*. Functional. Inside diameter (fixed preparations) 0·1–0·15 mm.

*Neck*. Approximately same diameter as scolex. Length very variable.

*Strobila*. Length 2·5–30 cm. or more.

*Young proglottids* 4·5 times broader than long.
*Mature proglottids.* 2·5 times broader than long.
*Gravid proglottids.* Approximately square or broader than long.
*Genital pore.* Irregularly alternating, approximately middle of margin.
*Cirrus-sac.* Length—quarter of breadth of mature proglottis.
*Ejaculatory duct.* Slightly coiled—very variable.
*Testes.* 60–75, rarely up to 90. Single layer.
*Vagina.* Opens anterior to cirrus; crosses cirrus-sac.
*Uterus.* Poral half—3–6, average 5. Aporal half—4–9, average 6–7.
*Host. Coregonus pollan.*
*Locality.* Lough Neagh, Ireland.

A consideration of the measurements of the length of the strobila and the neck, the diameter of the suckers and of various parts of the reproductive apparatus indicate that the dimensions of these structures are so variable that they cannot be taken as diagnostic of the species.

## THE MALE REPRODUCTIVE SYSTEM

*Testes.* The testes (Fig. 2, *T*) are round follicular bodies situated in the medullary parenchyma slightly nearer one surface which is thereby defined as dorsal. They are grouped in a single horizontal layer, bounded laterally by the ventral osmoregulatory canals. In a mature proglottis they occupy the entire area between the anterior border of the ovary and the anterior margin of the segment, being absent only from a small region in the immediate vicinity of the vas deferens.

The number of testes per proglottis varies between 60 and 75 with occasionally as many as 90. This number is greatly reduced in segments in which the uteri are distended with eggs and embryos. Here they tend to be confined to small areas in the vicinity of the vitellaria and are crowded together so that they are roughly polygonal in outline. In fixed material their diameter averages about 0·08 mm.

When mature, each testis consists of groups of tightly packed developing germ cells enclosed in a closely applied, delicate membrane in which occasional nuclei are scattered. In degenerating testes this membrane is greatly thickened and is no longer closely applied to the enclosed cells. A few parenchymal strands are always connected to the investing membrane and these appear to be continued into it.

*Vasa efferentia.* Each testis is provided with a single vas efferens. The latter are thin-walled structures generally visible only in microscopic sections. Their wall is composed of a very fine homogeneous membrane internal to which is a well-developed epithelial lining. The inner terminal portion of each vas efferens is continuous with the covering of the testis. The ducts of neighbouring testes anastomose before forming a network which opens into the vas deferens.

*Vas deferens, cirrus-sac, ejaculatory duct and cirrus.* The vas deferens (Fig. 2, *VD*) originates in the central region of the proglottis and continues in a transverse direction towards the base of the cirrus-sac, extending slightly beyond the anterior margin of the latter. It is extensively coiled so that it occupies most of the area between the dorsal and ventral transverse muscle sheets. It functions not

only as a seminal duct but as a storage reservoir and even in near-gravid segments is nearly always distended with large quantities of spermatozoa. The vas deferens terminates in the basal portion of the cirrus-sac (*CS*). Before entering the latter the diameter of the duct decreases and the muscle layer becomes thicker and more compact.

Indications of a very poorly developed epithelium lining of the vas deferens were occasionally evident, especially in the proximal portion of the duct. This appears to be replaced by a lining consisting of two layers; a thin inner layer and an outer, slightly thicker transparent layer. It was not possible to determine with certainty whether this lining is cuticle or a basement membrane; the former seems more likely. External to the lining is a fine layer of circular muscle fibres surrounded by a well-developed epithelium.

The cirrus-sac is rather short, having a length of approximately one-fourth that of the breadth of the proglottis. Its shape is extremely variable even in adjacent segments. It tends to be roughly pyriform in outline. In microscopic sections one or more constrictions are usually visible in the sac wall, especially in its extreme distal portion. These constrictions are better developed in the posterior margin of the sac and are frequently confined to the latter. Two conspicuous retractor muscles extend from the base of the sac and are continued on either side of the vas deferens to beyond the centre of the proglottis. In sections they are seen as bundles of transverse fibres which, in the central region of the proglottis, turn upwards towards the dorsal surface. A few of these fibres are inserted in the basal portion of the sac, while others appear to be continued into the proximal region of the ejaculatory duct. The wall of the sac is composed mainly of circular muscles; some longitudinal fibres are present, especially in the distal portion of the sac, and a few of these are continued for some distance into the parenchymal muscle layer. On the external surface of the wall there is a well-developed layer of epithelium and between this and the muscle fibres is a basement membrane. Another basement membrane is present on the inner surface of the muscle sheath. Internal to this is a lining of scattered, irregular epithelial cells which are sometimes difficult to distinguish as a separate layer. The cavity of the cirrus-sac is partly filled with a loose network of parenchyma, in which large cells are distributed. In size, shape and in respect of their spindle-shaped nuclei these cells closely resemble those of the subcuticular region. The distribution of these cells is uneven; they are present in groups almost completely confined to the areas surrounding the cirrus and the proximal portion of the ejaculatory duct.

The vas deferens penetrates the centre of the base of the cirrus-sac, to the inner surface of which it is attached by short, thick muscle fibres. Within the cirrus-sac the seminal duct becomes thick-walled and muscular. Strands of parenchyma connect it to the inner surface of the sac so that it is suspended asymmetrically within the latter, lying near the anterior margin. Its course is extremely variable even in adjacent segments.

The ejaculatory duct is sometimes an almost straight tube, but more usually it is coiled on itself. Its lumen gradually decreases in size from its proximal to its distal end. The lining of the duct appears to be similar to that of the vas deferens.

Its wall is muscular with inner circular and outer longitudinal layers. Initially the longitudinal fibres are arranged in separate, radiating bundles, between which are scattered parenchymal cells; distally, these fibres are arranged in a compact layer. Large cells resembling the subcuticular cells form a well-developed sheath external to the muscles. These are the so-called 'prostate cells'. They are frequently pyriform in outline and have narrow processes pointing towards the lumen of the duct. These processes, however, could never be traced further than midway in the longitudinal muscle layer. Frequently there are indications of an outer epithelial layer but this could not be distinguished with certainty from the surrounding 'prostate cells'.

*Cirrus*. The cirrus (*C*) is rather short and lies in the portion of the sac extending from the genital atrium to just beyond the region of the vitellaria. Laterally, its walls almost reach the inner surface of the cirrus-sac and are separated from the latter by strands of tissue packed with cells containing large, spindle-shaped nuclei. In the region where the ejaculatory duct becomes continuous with the cirrus there is a well-developed transverse sheet of parenchyma extending across the cavity of the sac. The distal end of the retracted cirrus is rounded. The outer portion of its wall consists of a layer of muscle fibres, many of which are inserted into the proximal region of the cirrus-sac. There is a well-developed cuticular lining in which two layers can be easily distinguished. That nearer the lumen is thin and stains deeply and is somewhat similar to the comidial layer of the external body cuticle. The cuticular lining in the marginal portion of the cirrus is very thin and is continuous with that lining the genital atrium. In the distal portion of the retracted cirrus it is much folded on itself so that it has a concertina-like appearance. The inner portion of the cuticle consists of a thick homogeneous layer which dips down into the folds of the lining. An external epithelium does not appear to be present.

An everted cirrus was rarely observed. When evertion occurs, the cirrus comes to lie in the genital atrium; its extreme tip, which was the proximal end of the retracted cirrus, becomes bluntly pointed and protrudes through the genital pore. The inner portion, which is now proximal, is continuous with the wall of the cirrus-sac. The cuticular lining of the cirrus is now the external covering, and under tension the folds disappear and it becomes smooth. A new cavity is formed in which lies the most distal portion of the ejaculatory duct. Since the entire ejaculatory duct is also under tension it assumes the form of a relatively thin-walled, straight tube, attached distally to the tip of the cirrus.

## THE FEMALE REPRODUCTIVE SYSTEM

*Ovary*. The ovary (Fig. 2, *O*) is a bilobed structure which lies in the posterior region of a proglottis and extends laterally to the vicinity of the terminal parts of the vitellaria (*YG*). Throughout the greater part of its length it usually closely approaches the posterior margin of the proglottis, but for a variable distance on each side of the middle line it narrows considerably, so that the two lobes are connected by an isthmus which increases in length and becomes narrower in mature proglottids. The ovary is enclosed by a membrane composed of irregularly spaced

flat cells each of which contains an elongate nucleus (Fig. 7, *WO*). Posterior to the isthmus and enclosed laterally by the lobes of the ovary are situated cells which appear to represent the shell or Mehlis's glands of other cestodes, and considerable lengths of the female genital ducts. The eggs, each containing a large nucleus and prominent nucleolus, are clearly visible in all parts of the ovary. In many of the proglottids examined the ova present in the isthmus and the median parts of the ovary are somewhat larger than those situated more laterally.

*Vagina, seminal receptacle and seminal canal.* In the present account the following terms are used. The duct lying between the genital atrium and the seminal receptacle is called the vagina, while that part of the female system which connects the receptacle with the oviduct is termed the seminal canal. The term uterine canal is used for the duct extending from the junction of the seminal canal and oviduct to the uterine pouches in the region of the proglottis anterior to the ovary.

The vagina (Fig. 2, *V*) opens into the genital atrium, anterior to the cirrus (*C*). It is continued towards the central part of the proglottis and crosses the cirrus-sac about the middle of the length of the latter. The point at which the vagina crosses the male duct is subject to variation, being frequently beyond the middle and sometimes close to the inner end of the sac. After the vagina has crossed the cirrus-sac, it continues inwards and downwards and, anterior to the ovary, enlarges to form the seminal receptacle (*SR*). The receptacle communicates with a narrow duct, the seminal canal (*SC*); the latter crosses the isthmus of the ovary, sometimes close to the middle line but frequently well to one side of the central region of the isthmus. The length and disposition of the seminal canal in the region of the proglottis posterior to the isthmus varies considerably. It usually forms one or more loops or curved regions before joining the oviduct and uterine canal. The vagina and part of the seminal canal anterior to the ovarian isthmus are not coiled.

The outer part of the vagina in the vicinity of the genital atrium is lined by cuticle which is at first closely similar to that of the atrium and of the outer surface of the proglottis (Fig. 3). Immediately beyond the vaginal sphincter muscle (*VS*) the cuticula becomes thicker and is clearly composed of an outer homogeneous region (*HL*) and an inner part (*CL*) which is optically identical with the comidial layer of the external cuticle. It is therefore proposed to extend the term comidial layer to include the inner part of the cuticular lining of the genital ducts. A thin layer of muscle (*ML*) is present in the wall of the vagina outside the homogeneous region of the cuticula. This is composed mainly of circular muscles but also contains a few longitudinal fibres. The layer becomes very thin in that part of the duct which is surrounded by the sphincter muscle but is continued almost to the outer limit of the sphincter. Both the cuticula and the layer of circular muscles extend as far as the junction of the vagina with the seminal receptacle. From the vicinity of the vaginal sphincter to its junction with the seminal receptacle, the vagina is surrounded by a layer of irregularly shaped cells with large nuclei (Figs. 3 and 4, *EC*). These cells resemble those of the parenchyma. They may be more than one layer thick in the immediate neighbourhood of the vaginal sphincter, but elsewhere are more regularly arranged so as sometimes to give the impression of an outer epithelial layer.

The vaginal sphincter (Fig. 3, *VS*) is seen to greatest advantage in thick sections. It varies in shape and may therefore be assumed to undergo movements which alter the diameter of the lumen of the vagina and presumably play a part in the transference of sperms from the neighbourhood of the genital atrium to the deeper parts of the vagina. The sphincter is composed of muscle fibres surrounded by a thin structureless membrane.

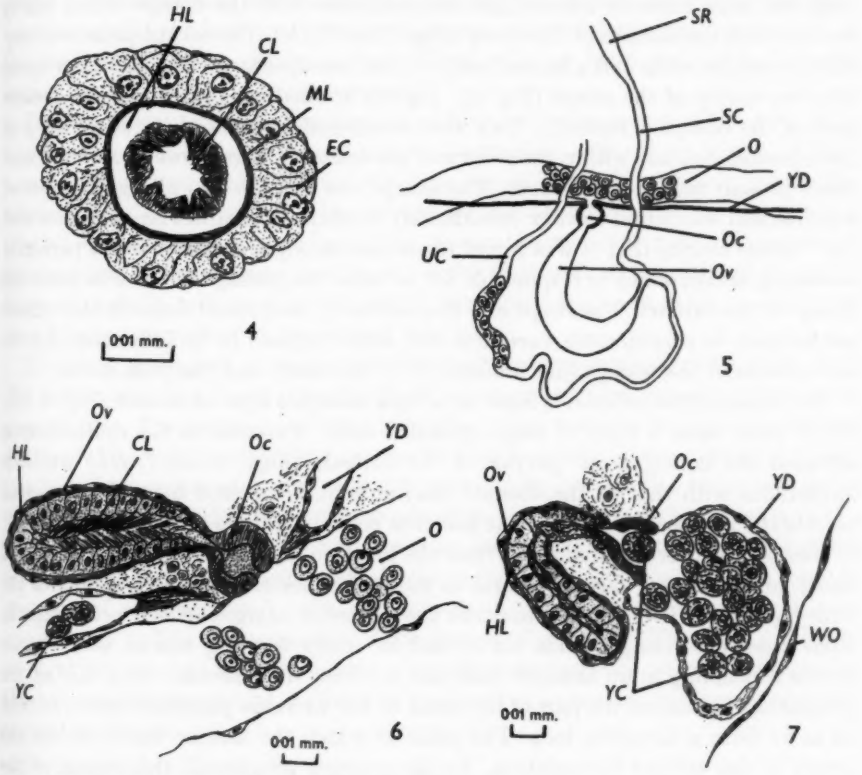



Fig. 4. Transverse section of the vagina.

Fig. 5. Diagrammatic drawing to show the oocapt, oviduct and adjacent parts of female reproductive system.

Fig. 6. To show the structure of the oviduct and the junction of oocapt and the ovary.

Fig. 7. To show the junction of the oocapt and the vitelline duct.

The lumen of the seminal receptacle is surrounded by a homogeneous cuticula without a comidial layer. The cuticula is surrounded by a thin layer of circular muscles which probably contain a few longitudinal fibres. Irregularly shaped cells, similar to those of the vagina, form the external layer of this part of the female system.

The part of the seminal canal adjacent to the receptacle is lined by a homogeneous cuticula. In the lower part of the canal this is replaced by epithelial cells, and the homogeneous cuticula comes to lie between the lining and the outer

covering of the canal. A muscle layer appears to be absent. A thin external layer of flat elongate cells surrounds the canal.

In some of the sections examined the vagina, seminal receptacle and canal contained numerous spermatozoa.

*Oviduct.* The oviduct (Figs. 2 and 5, *Ov*) arises from the junction of the seminal and uterine canals; it immediately expands into a wide-curved sac with thick walls but soon becomes narrow and communicates with the oocapt which lies in contact with the isthmus of the ovary (Figs. 2 and 5, *Oc*). The oocapt possesses very thick muscular walls and a lumen lined by thin homogeneous cuticula which opens into the cavity of the ovary (Fig. 6). Lightly stained cells surround the greater part of the oocapt externally. Ova were occasionally observed in the vicinity of the opening, but not within the cavity of the oocapt. Eggs, however, were sometimes present within the oviduct. The oocapt was identified in a large number of sections and was found to vary considerably in shape. Its muscular character and the various shapes that it was found to assume strongly suggest that it performs a sucking action which is responsible for, or aids, the passage of the ova from the ovary to the oviduct. The shape and disposition of the genital ducts in this region are subject to considerable variation and would appear to be influenced by the movements of the oocapt and pressure from the ovary and the yolk ducts.

The lumen of the oviduct is lined by a thick comidial layer of cuticle (Fig. 6, *CL*) which rests upon a layer of large epithelial cells. External to the epithelium is situated the homogeneous portion of the cuticula (Figs. 6 and 7, *HL*) which is continuous with that of the oocapt; this, in turn, is covered by a thin external membrane composed of rather flat elongate cells. A muscle layer is absent.

*Uterus.* The uterine canal arises from the junction of the oviduct and the seminal canal, and follows a variable course in the region posterior to the isthmus of the ovary (Fig. 5, *UC*). It runs towards the posterior margin of the proglottis, in some cases describing a wide curve and in others forming one or more bends before continuing in an anterior direction to cross the isthmus. In a few of the proglottids examined the part of the canal in the extreme posterior region is bent so as to form a complete loop. The point at which the uterine canal crosses the ovary is also subject to variation. In the younger proglottids this region of the female genital tract is not clearly differentiated, but as growth proceeds its walls become sharper and it may be followed into the tissues immediately anterior to the ovary.

As the proglottis matures the uterine canal increases in width, and at this stage is clearly seen to possess a fairly thick wall composed of epithelial cells covered by a homogeneous cuticula and a thin external cellular membrane. Ova were frequently visible in this part of the tube. Later, the uterus increases in length and spreads in an anterior direction as a wide tube with thin walls which occupies the central part of the proglottis. Finally, it gives off lateral pouches on each side which may in turn possess smaller lateral branches (Fig. 2, *U*). The greater part of a gravid proglottis, therefore, is occupied by the uterine pouches packed with developing ova and early embryos. In gravid proglottids the number of uterine pouches on the side opposite to the genital pore varies between four and nine with

an average of six to seven per proglottis. On the side bearing the genital pore the number varies between three and six, with an average of five per proglottis (Fig. 2).

*Mehlis's gland.* This gland is poorly developed and consists of a few elongate cells in the neighbourhood of the oocapt and the adjacent region of the oviduct.

*Vitellaria.* The vitellaria (Fig. 2, *YG*), or yolk glands, lie lateral to the testes and uterus and on each side extend almost to the ovary. The vitelline cells are continuous throughout the length of the glands, except where interrupted, on one side of the proglottis, by the genital atrium. These cells are large and contain deeply stained nuclei and extra-nuclear material.

*Vitelline ducts.* Owing to the thinness of their walls, the course of the yolk ducts is, except in very favourable preparations, extremely difficult to follow. A duct runs from the posterior limit of the vitellaria on each side and very soon crosses the ovary in a diagonal direction to emerge in the neighbourhood of the oviduct (Figs. 2, 6 and 7, *YD*). The course of the terminal part of each yolk duct is subject to variation. In some cases it approaches the wide part of the oviduct and then bends sharply to terminate in association with the oocapt. In other cases it runs close to the ovary after it has crossed the latter.

The walls of the yolk ducts consist of flat cells with prominent nuclei (Figs. 6, 7). The lumen may be greatly extended by the presence of vitelline cells or it may be very narrow. The manner of the termination of the ducts in connection with the cavity of the oocapt is difficult to determine with certainty. Examination of a large number of preparations, however, leaves little doubt that the two transverse ducts unite and immediately open into the oocapt in the vicinity of the junction of the latter with the cavity of the ovary. In some few cases the parts of the yolk ducts in the immediate neighbourhood of the oocapt were greatly enlarged by the presence of numerous yolk cells, and in two of the sections examined yolk cells were clearly visible in the opening of the oocapt (Fig. 7, *YC*). The ducts appear to be highly elastic and when distended with yolk cells exert considerable pressure on the surrounding structures, so that the oviduct and ovary are displaced from their normal positions.

## DISCUSSION

Benedict (1900) gave detailed descriptions and figures of the female reproductive system of *P. ambloplitis* and of a species he believed to be *P. filicollis*. La Rue (1914), however, considered that the latter was *P. exiguus*. Benedict, and later La Rue, recorded the presence of a strong vaginal sphincter situated close to the genital atrium of *P. ambloplitis*. It is worthy of note that both these authors stated that the vagina of *P. ambloplitis* opens into the oviduct, and that Benedict remarked that 'further investigations will probably show that in *Proteocephalus* the vagina usually opens into the oviduct and not into the ootype'. More recently, Meggitt (1914), MacLulich (1943) and Alexander (1951) have shown that in *P. filicollis*, *P. parallacticus* and *P. salmonidicola*, respectively, the vagina joins the oviduct. The present findings on the relationships of these ducts in *P. pollanicola* is in agreement with the accounts cited above. The writers found that the course of the

seminal canal (often referred to as the vagina) and of the oviduct and uterine canal varies considerably in different proglottids from the same worm. This tendency was noted by Benedict who remarked that the course of the oviduct of *P. ambloplitis* was never exactly the same.

The vaginal sphincter is a prominent feature of the female reproductive system of *P. pollanicola*. This structure appears to be poorly developed in some proteocephalids and is not recorded as present in all species which have been examined.

Wardle & McLeod (1952) stated that the function of the oocapt has not been determined with certainty. It is believed to undergo 'rhythmic movements, expanding to admit an ovum from the ovary and contracting to force it farther down the tube'. They point out, however, that Johnstone believed that the ova were expelled from the ovary by the contraction of the muscles of the proglottis, and that the oocapt is an elastic sphincter. The present observations strongly support the view that the oocapt undergoes rhythmic movements which assist the passage of the ova from the ovary to the oviduct. The writers believe that the oocapt also receives vitelline cells from the transverse yolk ducts. The observation that the yolk ducts open into the cavity of the oocapt appears to be new. Previous workers who have followed the yolk ducts of proteocephalids record their junction with the female canal at or close to the ootype. It has also been claimed that the two transverse yolk ducts unite to form a common duct which in turn communicates with the female genital canal. In *P. pollanicola*, the transverse yolk ducts unite in the immediate vicinity of the oocapt and a long common duct is not present.

Many workers record the presence of an ootype in the female reproductive system of cestodes. According to Wardle & McLeod the 'U-shaped region, where the oviduct bends upward and forward, is termed the "ootype", or primary uterus. It is surrounded by numerous elongated, club-shaped cells, the so-called "shell-glands" or Mehlis's glands of early descriptions.' They state that it seems to be well established that these gland cells produce a secretion which is passed into the cavity of the ootype. During the present investigation a specialized region of the oviduct corresponding to the ootype of other workers was not identified. A few elongate club-shaped cells closely resembling those of Mehlis's gland of other cestodes were present in the vicinity of the oocapt. These gland cells do not form a compact mass. Their disposition and arrangement indicates that their secretion is discharged into the cavity of the oocapt or adjacent region of the oviduct. As far as the writers are aware unicellular glands have not previously been described as occupying a similar position in any proteocephalid.

The present observations on the histological structure of the vagina, seminal receptacle and seminal canal of *P. pollanicola* differ in some respects from previous accounts. Benedict believed that the cuticle from the external surface was continued into the vagina for some distance beyond the vaginal sphincter. It was, he stated, not possible to trace the transition from the cuticle to the epithelial layer present in the deeper parts of the vagina. He did not describe in detail the structure of the vaginal wall of *P. ambloplitis* but stated that in *P. filicollis* a thin layer of muscle fibres is present outside the ciliated lining and that the muscle layer is

surrounded by a delicate epithelium. He figured the 'epithelial layer' as devoid of nuclei, and later Meggitt showed a similar condition for the vagina of *P. filicollis*. Later, it was claimed that the epithelium lining the canals of the female reproductive system of cestodes is frequently 'replaced by a cuticle, often hairy or fringed' (Hyman, 1951). Some workers believed that the lining of parts of the ducts at least are provided with cilia (Wardle & McLeod). The present observations support the view that regions of the genital ducts when fully developed are lined with cuticle. The writers consider that cilia are not present in the female ducts of *P. pollanicola*, but admit that as the inner margin of the comidial layer is usually faintly stained and sometimes broken, the striations frequently give the impression of a ciliated lining. It is evident that the non-nucleated 'epithelium' described by some previous workers is in reality the homogeneous region of the cuticula, and the 'cilia' the striations of the comidial layer. The present observation that the comidial layer of the oviduct is separated by epithelial cells from the homogeneous region of the cuticula appears to be new.

Wardle & McLeod state that, as in other flatworms, the vas deferens of cestodes is ciliated. No trace of cilia was found in any part of the male reproductive apparatus of *P. pollanicola*.

It appears to be generally accepted that muscle fibres are present in the wall of the seminal canal (Wardle & McLeod). The present writers were unable to identify fibres in this part of the female reproductive system of *P. pollanicola*. It is of interest that Meggitt stated that just prior to the junction of the vagina and oviduct the walls of the former diminish in thickness and that the 'muscular layer nearly disappears'.

In view of the probability of considerable variation in the course, and perhaps in the structure, of the female reproductive canals of different cestodes there is little point in discussing further the conclusions of previous workers.

Benedict described and figured a layer of gland cells with long processes directed towards the lumen of that part of the vagina which lies in the neighbourhood of the vaginal sphincter. He also believed that gland cells are present in the wall of the seminal canal, oocapt and oviduct of *P. ambloplitis*. Meggitt stated that gland cells with fine ducts are situated in the wall of the vagina but are absent from the oviduct of *P. filicollis*. During the present observations it was found that the nuclei of the large cells external to the muscle layer of the wall of the vagina are sometimes somewhat pointed. Occasionally deeply stained material lies between the pointed end of a nucleus and the lumen of the vagina. With a $\frac{1}{6}$ in. objective, these nuclei and the adjacent deeply stained material might be mistaken for a layer of unicellular glands. Careful study with a $\frac{1}{12}$ in. oil-immersion objective shows that these cells closely resemble those situated on the external surfaces of other parts of the female reproductive system, and the large cells present in the subcuticular region of the proglottids.

Benedict described a layer of pyriform glandular cells external to the muscular wall of the ejaculatory duct of *P. ambloplitis*. These possess long processes which project into the muscles but could not be traced beyond this layer. Meggitt recorded numerous pear-shaped glands which he described as opening into the

lumen of the cirrus. La Rue believed that the presence of cells, which he called 'prostate gland cells', is characteristic of the ejaculatory duct. 'Prostatic cells' have been frequently described in other cestodes, but Young (1913) considered that these cells are 'in no way different from those of the parenchyma'. In the authors' opinion these cells, like those surrounding the female genital ducts, resemble the cells of the subcuticula and not the ordinary parenchymous cells. In *P. pollanicola* they do not communicate directly with the cavities of the genital ducts. However, it is conceivable that they may produce a secretion which permeates through the inner layers.

According to Benedict the oocapt of *P. ambloplitis* is lined by cubical epithelium surrounded by circular muscle fibres. Meggitt, however, observed that this organ is lined with 'simple epithelium' and figured muscle fibres as forming the greater part of its wall. In *P. pollanicola* the wall of the oocapt is composed almost wholly of muscles and its lining is a continuation of the homogeneous layer of the cuticula of the oviduct. Meggitt recorded the presence of 'an irregular ring of nuclei' surrounding the oocapt. It is probable that these are the nuclei of the lightly staining cells observed during the present observations.

## SUMMARY

An account is given of the general structure of *Proteocephalus pollanicola*. The genital system is dealt with in greater detail. The characteristic features of the species are enumerated.

The male portion of the reproductive system consists of 60–75 testes in a single layer; vasa efferentia with a lining epithelium; a wide, coiled vas deferens; a small cirrus-sac; a coiled ejaculatory duct and a short cirrus. A description is given of the histological structure of the male ducts.

The vagina opens anterior to the cirrus-sac. A well-developed vaginal sphincter is present. The seminal canal communicates with the oviduct and the uterine canal. An oocapt is present; the transverse vitelline ducts and the ovary open into it. A few unicellular glands are present in the vicinity of the oocapt; these possibly represent Mehlis's gland. An ootype is absent. The histological structure of the female ducts is described.

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# AN INVESTIGATION ON THE LIFE CYCLE OF *TRICHURIS MURIS*

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(With 7 Figures in the Text)

## INTRODUCTION

Data concerning the life cycle of the various species of the genus *Trichuris* are somewhat controversial. The present paper is devoted to the study of *T. muris*, a common parasite of the caecum of wild house-mice and rats. The possibility of transfer of *T. muris* in the laboratory mice would enable the author to solve various contradictory points.

Davaine (1858) was the first author to deal with the life cycle of *T. trichiura*. He pointed out that the eggs are passed in a single cell stage, and when kept in water required a period of 8½ months to develop embryos. On the other hand, Leuckart (1876) found that a period of 4–5 months is sufficient for the eggs to develop larvae. He also found that the life cycle of *T. trichiura* does not require an intermediate host. He infected a lamb with embryonated eggs of *T. ovis*, and in a post-mortem examination after 16 days, he found the larvae in the caecum, while with *T. crenatus* in the pig, he found almost mature specimens in the caecum after 4 weeks.

That the infection can be produced only when the eggs are embryonated was shown by Railliet (1884) in his experiments on dogs fed with embryonated and unembryonated eggs of *T. vulpis*. Examination of dogs killed 3 months after infection revealed that those fed with embryonated eggs contained numerous mature specimens of *Trichuris*, while dogs fed with unembryonated eggs did not show any sign of infection.

Neshi (1918) dealt with *T. vulpis* in dogs, and reported that this species undergoes a blood migration to the lungs. Other workers failed to confirm this view. Tochihara (1922), who studied the development of *T. vulpis* in dogs and rats, found that the eggs hatch in the stomach and small intestine an hour after ingestion; and that the larvae remain in the intestine till they reach maturity without any evidence of migration through the lung. This was also confirmed by Fülleborn (1923) who infected three guinea-pigs and a rat with embryonated eggs of *T. trichiura*. He also succeeded in infecting a rabbit with eggs of *T. leporis* from a hare, and a monkey (baboon) with eggs of *Trichuris* (which he suspected to be *T. trichiura*) from another monkey's faeces.

Matsubayashi (1932) found that the larvae of *T. vulpis* in their development after hatching, penetrate the mucous membrane of the small intestine, where they stay temporarily for a period of from 6 to 9 days, later appearing in the lumen.

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No further reports on their development were possible, since he failed to locate them after this time. These results disagree with those of Tochihara (1922) who found that the larvae of *T. vulpis* remain in the intestine until they reach complete development.

Finally, Miller (1947) on carrying out some experiments on the life cycle of *T. vulpis* in dogs confirmed that there is no blood migration. According to him, the larvae penetrate the mucous membrane of the upper part of the small intestine within 24 hr. after infection, and become deeply embedded at the base of the crypts of Lieberkuhn. About the 10th day, the larvae begin to leave the mucous membrane and are carried to the large intestine, where they reach maturity. Miller did not observe any moult of the larvae within the egg from the 1st to the 10th day of the larval development in the intestine.

## SOURCE OF MATERIAL

A quantity of eggs of *T. muris* were recovered from the faeces of infected house mice, *Mus musculus*, obtained from the bird house in the Zoological Park in Edinburgh. These were not sufficient to carry out the experiments on the life cycle, so more mice were trapped, and female worms from them were dissected yielding abundant uterine eggs.

## INCUBATION OF THE EGGS

The eggs were cultured in normal saline solution (0·85 % NaCl) or in distilled water or in tap water. Quantities of eggs were placed in watch-glasses, each covered with a small amount of one of these fluids to a depth up to 5 mm. To prevent bacterial growth, a few drops of 5 % formaldehyde solution were added to each of these cultures which were incubated at a temperature of 25–26° C., and the eggs were examined daily for evidence of development. Care was taken to prevent evaporation by periodic addition of the fluid.

The first outlines of the larvae appeared in 22–23 days and the fully grown larvae in 30–31 days of incubation (Fig. 1). The embryonated eggs were now examined daily for moulting. In spite of using oil-immersion and phase-contrast microscopes, the moult of the larvae within the eggs was not observed, and the larvae were seen to be motile within the shells. Since the time for development of the eggs was found to be prolonged, the following experiments were carried out in an attempt to accelerate it.

Fig. 1. *T. muris*, embryonated egg.

Eggs placed in separate dishes containing different solutions were incubated at a temperature of 25–26° C. On successive examination of the eggs every 24 hr., it was found that those incubated in the 0·25 % sodium bicarbonate solution develop larvae inside their shells after 26 days, while the eggs incubated in N/50-HCl, N/25-HCl and 0·5 % sodium bicarbonate did not show any further development beyond the 6th day when the cleavage ceased.

Eggs of *T. muris* incubated in distilled water, tap water, and normal saline solution at a temperature of 22 and 37° C. failed to develop embryos. In the eggs incubated at 22° C., the development proceeded only until the 14th day, while in those incubated at 37° C., cleavage of the embryonal cells stopped at about the 4th day.

The above results indicate that the eggs do not develop in acid media, while in weak alkaline media (0·25 % sodium bicarbonate solution) at 25–26° C., which is the optimum temperature, they develop embryos in 26 days (2 days later than those incubated in normal saline solution, distilled water or tap water).

## INFECTION OF LABORATORY MICE

The laboratory mice used in these experiments were 21–25 days old. They were infected with the embryonated eggs 2 weeks after the larval formation had started. The mice were starved for 3 hr. and fed with bits of bread upon which were spread infective eggs, and no water was given to the mice for 2 hr. after infection. In this way, twenty-five mice were infected and, at certain intervals, one by one they were killed for examination of the alimentary tract for the larvae of *Trichuris*. The mucous membrane was examined in the first two mice in order to find whether the larvae were migrating in the mucous membrane. For this purpose small pieces of intestine were placed between two glass slides, and carefully examined. In both cases the larvae were found in the lumen of the small intestine on their way to the caecum or had already arrived there. Besides the examination of the mucous membrane, the intestinal contents were also carefully examined in order to locate the larvae and to determine the length of time they had taken to travel along the alimentary canal to their final habitat. The results of these experiments are shown in Table 1.

Daily examination of the faeces of four other infected mice revealed the presence of the eggs after 34–35 days from time of infection.

## DISCUSSION

Several authors have dealt with the embryonation of the genus *Trichuris* (Davaine, 1858, 1863; Brown, 1927; Splinder, 1929; Nolf, 1932; Onorato, 1932; Dinnik & Dinnik, 1937; Miller, 1939). Those who have dealt with *T. trichiura* have stated that the eggs are readily affected by strong sunlight, by the extremes of temperature and by lack of moisture. Nolf (1932) has shown that the eggs of *Trichuris* from humans are more susceptible to desiccation and to heat and cold than the eggs of *Ascaris* from the same source. The former died when the temperature fell below −9° C. or rose above 52° C.

Brown (1927) found that in *Trichuris trichiura* under favourable conditions of temperature and humidity in shady sand or clay, larvae developed in 33 % of eggs in 21 days and in 67 % in 35 days. According to Miller (1939), almost 100 % of the eggs of *T. vulpis* subjected to centrifugation and to immersion in brine for 5 min. were completely developed in 13–14 days respectively. Eggs submitted to both these treatments required only 9 days' incubation. Deo (1947) reported that the eggs of *T. ovis* cultivated in aerated distilled water developed larvae in 13–15 days.

All these authors agreed that the development of *Trichuris* eggs requires adequate moisture and moderate temperature. Furthermore, they have shown that the eggs of different species of the genus *Trichuris* differ in the time necessary for their development.

Table 1

(Period of infection = interval between infection and post mortem.)

| Mouse no. | Period of infection | Position of parasite | Stage and number of parasites | Larvae: Length (mm.) | Larvae: Width (mm.) | Remarks |
|---|---|---|---|---|---|---|
| 1 | 1 hr. | Stomach | Eggs, embryonated and non-embryonated | — | — | Rest of alimentary tract free from eggs |
| 2 | 2½ hr. | Lower part of jejunum and ileum | Free larvae, empty shells, unembryonated eggs | 0·16–0·162 | 0·01 | Larvae sluggish (Fig. 2) |
| 3 | 24 hr. | Caecum | 22 larvae | 0·189 | 0·012 | Anterior part of larvae embedded in mucous membrane |
| 4 | 2 days | Caecum | 10 larvae | 0·202 | 0·012 | |
| 5 | 3 days | Caecum | 16 larvae | 0·206–0·212 | 0·014 | — |
| 6 | 5 days | Caecum | 14 larvae | 0·24 | 0·016 | Oesophagus 0·168 mm. long |
| 7 | 7 days | Caecum | 10 larvae | 0·382 | 0·022 | — |
| 8 | 9 days | Caecum | 12 larvae | 0·45 | 0·023 | Oesophagus and intestine more pronounced (Fig. 3) |
| 9 | 11 days | Caecum | 8 larvae | 0·65 | 0·027 | Oesophagus 0·49 mm. long—six larvae were moulting |
| 10 | 13 days | Caecum | 8 larvae | 0·98 | 0·031 | — |
| 11 | 15 days | Caecum | 12 larvae | 1·3 | 0·036 | — |
| 12 | 17 days | Caecum | 8 larvae | 2·7 | 0·06 | Oesophageal cells distinct (Fig. 4) |
| 13 | 19 days | Caecum | 10 larvae | 3·4 | 0·066 | Oesophagus 2·51 mm. long |
| 14 | 21 days | Caecum | 9 larvae | 4·43–6·1 | 0·09–0·1 | Oesophagus 3·51–4·4 mm. long |
| 15 | 23 days | Caecum | 9 larvae | 5·8–9·52 | 0·14–0·16 | Larvae were moulting for the second time (Fig. 5) |
| 16 | 25 days | Caecum | 7 larvae | 6·9–10·5 | 0·15–0·17 | Larvae in two groups corresponding to future males and females |
| 17 | 27 days | Caecum | 7 young males and females | ♂ 8·8–10·1<br>♀ 14·2–15·8 | 0·162<br>0·178 | Male and female reproductive organs well developed (Figs. 6, 7) |

In the course of this study, it was found that at 25–26° C. and adequate moisture, larvae develop in 23 or 24 days.

Contrary to the findings of Miller (1939), saline was not found to accelerate the development of the larvae within the eggs of *T. muris*. On the other hand, there was no development of the eggs in acid media, but the eggs which were immersed in weak alkaline solution (0·25% sodium bicarbonate) matured, although more slowly than in normal media.

Infective eggs of *T. muris* (containing fully developed larvae) fed to mice, failed to hatch in the stomach. This is contrary to the findings of Tochihara (1922), but it agrees with those of Matsubayashi (1932) and Miller (1947) who pointed out that the larvae in *T. vulpis* hatch in the small intestine.

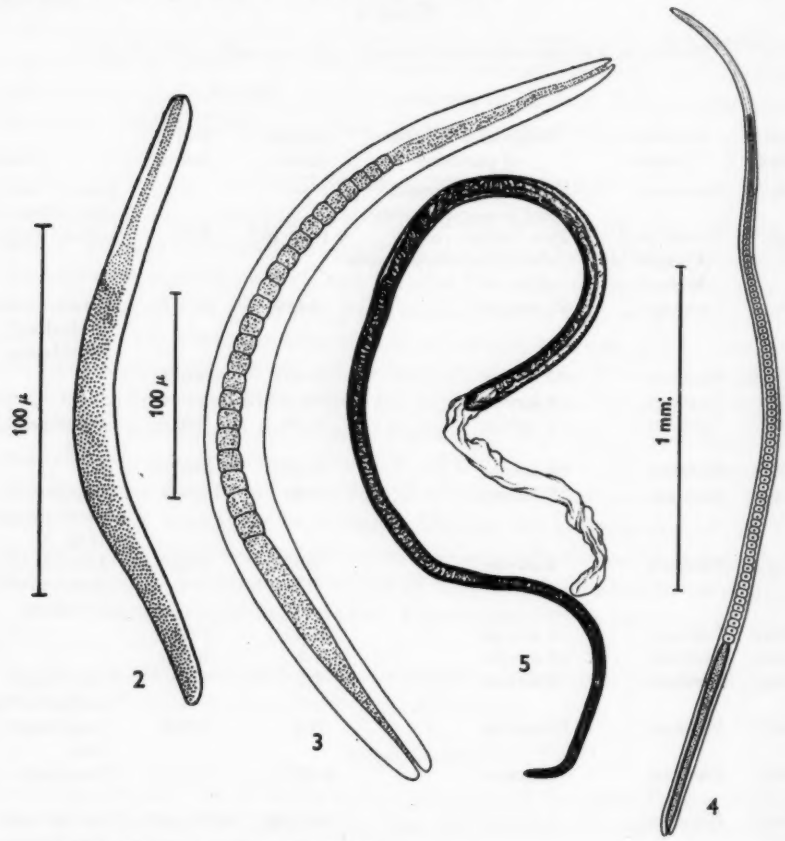


Fig. 2. *T. muris*, newly hatched larva.
Fig. 3. *T. muris*, immature, 9 days after infection.
Fig. 4. *T. muris*, immature, 17 days after infection.
Fig. 5. *T. muris*, larva in process of moulting, 23 days after infection.

It was also found that the eggs of *T. muris* remain in the stomach with the food for a period of not less than an hour (Table 1). In the mouse killed 2½ hr. after infection, the food contents had moved from the stomach to the small intestine, mainly to the jejunum and ileum, while some had reached the caecum. In the lower part of the jejunum, eggs were found in the process of hatching, and free larvae were present in the lumen. It is possible that some of these larvae had hatched in the upper part of the small intestine. The larve leave the egg shells through one of the plugs.

Neshi (1918) (quoted by Yokogawa, 1921) claimed that lung migration takes

place in *T. vulpis*. Fülleborn (1923), Hasegawa (1924), and Miller (1947) refuted this, and the findings of the present investigation confirm the latter results. The larvae of *T. muris* were found in the caecum 2½ hr. after infection, and they remained there until maturity; no lung migration taking place.

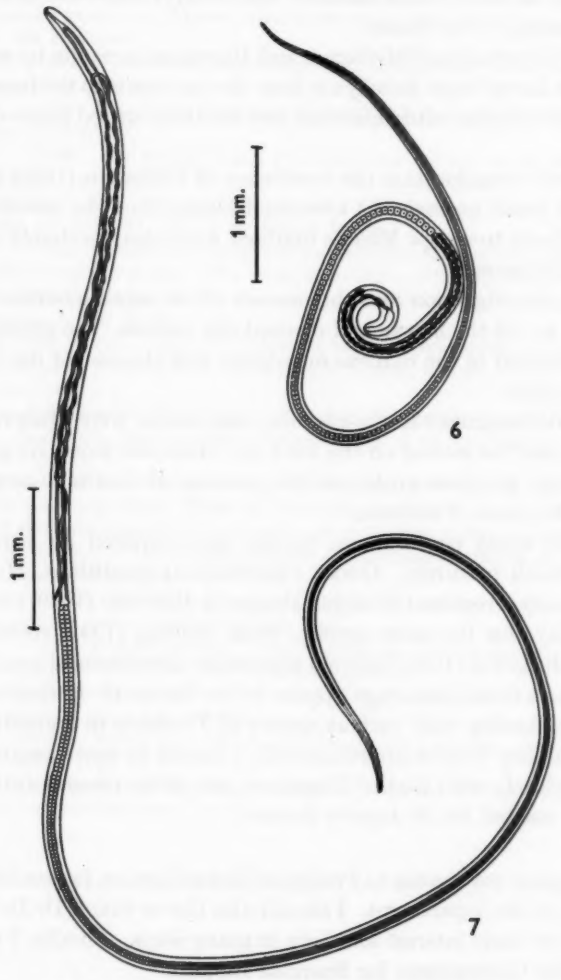


Fig. 6. *T. muris*, male, 27 days after infection.
Fig. 7. *T. muris*, female, 27 days after infection.

The detailed experiments carried out by Fülleborn (1923) show that in guinea-pigs infected with eggs of *T. trichiura* (from human source) all the larvae had reached the caecum after 24 hr., while in rabbits this stage is reached in less than 2 days. Similar results were obtained by him in rabbits fed with eggs of *T. leporis* and the eggs of *Trichuris* of monkey origin.

Hasegawa (1924) reported that eight young dogs infected with *T. vulpis* (*Trichocephalus depressiusculus*) the larvae hatched in the lumen of the small intestine and penetrated into the villi where they stayed for 2 days. Some of them were found in the caecum 70 hr. after infection. He also found that the time required for the larvae to reach maturity was 36 days after infection when the eggs were found passing in the faeces.

Miller (1947) contradicts Fülleborn's and Hasegawa's results by stating that the majority of the larvae begin to migrate from the mucosa into the lumen of the small intestine on the 10th day after infection, and are then carried passively down to the large bowel.

By taking into consideration the conclusion of Fülleborn (1923) for *T. trichiura* and *T. leporis* (with normal and abnormal hosts), and the results given in this paper, it is difficult to accept Miller's findings, which may probably be due to some unusual circumstances.

In *T. muris*, no migration into the mucosa of the small intestine was observed, and within 24 hr. all the larvae had reached the caecum. The anterior end of each larva was embedded in the mucous membrane and the rest of the body was lying close to the surface.

During the development in the caecum, two moults were observed, the first on the 11th day, and the second on the 23rd day after infection. No report has been published by any previous worker on the presence of moults in any of the species belonging to the genus *Trichuris*.

There is still much confusion as to the time required by various species of *Trichuris* to reach maturity. Under experimental conditions, Hasegawa (1924) found that *T. vulpis* required 36 days. Morgan & Hawkins (1949) stated this period to be 70–90 days for the same species, while Belding (1952) records 90 days for *T. trichiura*. Mozgovoi (1952) infected pigs under experimental conditions with the same species and found that eggs appear in the faeces 45–47 days after infection. Mönnig (1947), dealing with various species of *Trichuris* in domesticated animals, maintains that they require approximately 1 month to reach maturity. Mönnig's record agrees closely with that of Hasegawa and of the present author, who found that *T. muris* needed 34–35 days to mature.

I wish to express my thanks to Professor Michael Swann for enabling me to carry out this study in his department. I should also like to thank Dr D. O. Morgan and Dr C. Rayski for their interest and help in many ways. Finally, I owe my thanks to the Egyptian Government for financial support.

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# THE EFFECT OF CORTISONE ON *PLASMODIUM BERGHEI* INFECTIONS

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(With 4 Figures in the Text)

## INTRODUCTION

With the increasing use of cortisone in clinical medicine and experimental work, there has been found to be a considerable danger of aggravating or re-activating underlying infections. This has been observed with various bacterial and virus diseases, including the human varicella virus and poliomyelitis; it seems probable that in human malaria an acute attack might be precipitated in this way. Other infections, by plasmodia and trypanosomes, have been found to show similar reactions to the administration of cortisone.

Schmidt & Squires (1951), working on *Plasmodium cynomolgi* in *Rhesus* monkeys, found that 10 mg./kg. of cortisone daily starting 2 days prior to infection greatly intensified the parasitaemia during the post-crisis stage, while 10–50 mg./kg. in the chronic or latent stages produced severe recrudescences. No proliferation of macrophages was observed in the spleen, and the effects produced were said to be very similar to those of splenectomy. Redmond (1952), working with *P. relictum* in pigeons, gave 5 mg. cortisone daily starting 1–3 days before inoculation. This author found that few cortisone-treated animals showed a higher initial peak of parasitaemia, but there was a steep rise later and death usually resulted, whereas control animals survived and parasites were not detectable. Cortisone was said to interfere with the production of immunity, and not to affect the parasites, as paludrine controlled the infection in the experimental cortisone-treated animals. During latency, when an immune reaction was in operation, no relapse was produced by the administration of cortisone. Shwartzman & Fisher (1952) studied the effect of cortisone on the poliomyelitis virus in Syrian hamsters, and found that the infected hamster was very much more susceptible to cortisone than was the mouse. Most hamsters died, with much more severe illnesses than were observed in the mice, where only a third failed to survive. These workers found that the timing of the cortisone treatment was important. 3 mg. immediately following inoculation gave a greatly increased mortality rate with a much diluted virus, but even 5 mg. produced only a moderate increase when given 18 hr. before, 18 hr. after, or 2 hr. before inoculation. A much more concentrated virus preparation was needed for doses given with this timing to have a marked effect on the survival rate.

Findlay & Howard (1952) have performed an interesting series of experiments on mice infected with *P. berghei*. Mice received a total of 15 mg. cortisone acetate in three doses: 30 min. before inoculation with infected blood, 6 hr. later, and 4 days

later, while control mice received similar doses of saline. Blood films were taken from the tail and were stained with Giemsa. In the cortisone-treated mice parasitized cells appeared in the peripheral blood much earlier than in the control mice. In 72 hr. after infection 300 cells per 1000 were parasitized in the experimental mice, but a similar level of parasitaemia was reached in the control mice only 7 or 8 days after inoculation. The experimental mice died in about 5 days whereas the control mice died in 9–10 days. It was suggested that cortisone had inhibited the cellular response to infection. The level of dosage in these experiments was very much higher than in those of Schmidt & Squires (1951), Redmond (1952), and Shwartzman & Fisher (1952).

In the present work the experiments of Findlay & Howard have been repeated, using mice infected with *P. berghei* and treated with similar doses of cortisone, but the results obtained have not confirmed their observations.

## TECHNIQUE

Two strains of *P. berghei* have been used in this work. One was an extremely feeble, attenuated strain: the 'RSVP' strain, which had been kept in laboratory animals for 10 months and which had after a short time lost its gametocytes. The other strain was a much more virulent one, the 'C' strain, with numerous gametocytes. This strain was obtained from the Belgian Congo by courtesy of Dr Vincke, and had been kept for 4 months in the laboratory. Both strains had been kept by blood passage at frequent intervals in young hamsters.

The cortisone used in this work was produced by Merck & Company, Inc., and was kindly given by the Medical Research Council.

Mice weighing about 20 g. were infected with *P. berghei* by intraperitoneal inoculation of citrated hamster blood. Experimental animals were given 5 mg. of cortisone acetate intraperitoneally 30 min. before inoculation, 6 hr. after inoculation, and 4 days later. This was the same timing as that used in Findlay and Howard's work. Control animals received similar doses of saline.

Findlay & Howard used the intramuscular route for cortisone injections, but since no difference was found in preliminary experiments between that and the intraperitoneal route, the latter method has been used throughout the work.

## RESULTS OF EXPERIMENTS CARRIED OUT ON MICE

Blood films were taken from the tails, stained with Giemsa in the usual way and examined daily. Parasitized red cells were counted and expressed as numbers per microscope field (oil immersion, 10 eye-piece). The results which are summarized in Figs. 1 and 2 show that there is little difference in the level of parasitaemia in the experimental and control mice for the same strain of parasite. The mice of both groups lived for a considerable time showing increasing anaemia, with 40–50 % of the cells parasitized. Most of the cells were young forms.

The length of survival of some of the more typical cases is shown in Table 1. Others were killed for post-mortem examinations and some died but the immediate cause of death was not considered to be malaria.

A variation in the timing of the cortisone dosage was tried in a group of six mice, 5 mg. being given 3 days before inoculation, on the day of inoculation, and 2 days later, but no difference could be detected between experimental and control animals.

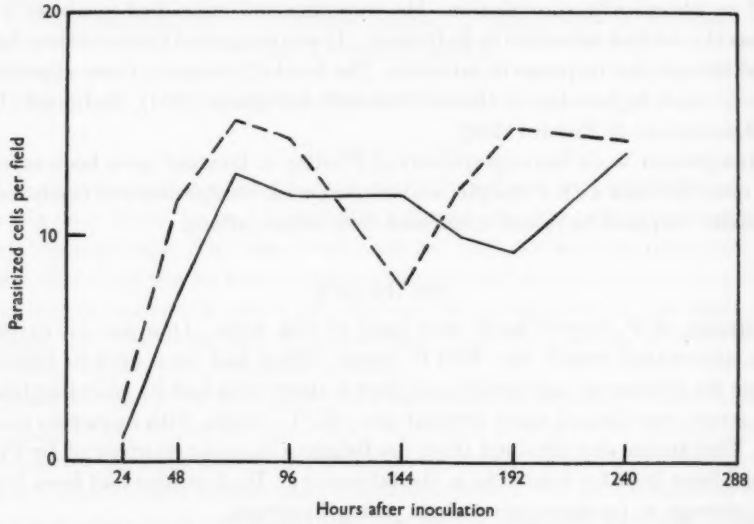


Fig. 1. Course of a *P. berghei* infection in mice treated with cortisone ('C' strain). All points are averages of five mice. ——, control mice; - - - -, cortisone-treated mice.

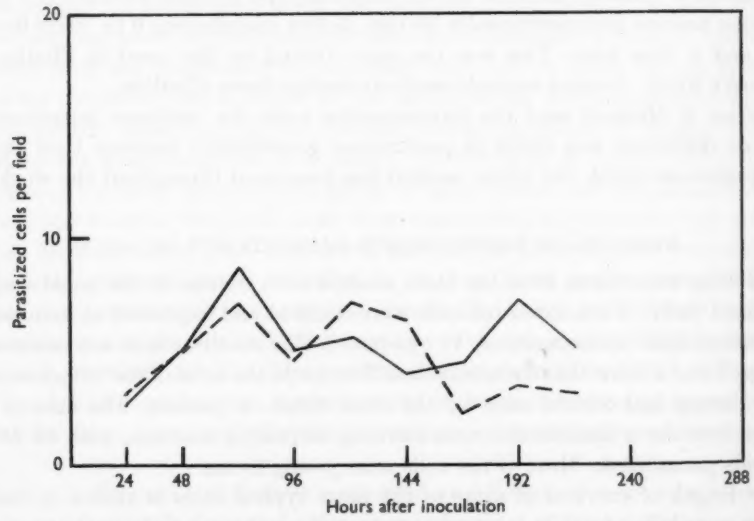


Fig. 2. Course of a *P. berghei* infection in mice treated with cortisone ('RSVP' strain). All points are averages of five mice. ——, control mice; - - - -, cortisone-treated mice.

Table 1

| Mouse no. | Survival in days | Strain |
|---|---|---|
| 13, Exp. | 32 | RSVP |
| 14, Exp. | 28 | RSVP |
| 15, Exp. | 25 | RSVP |
| 18, Control | 28 | RSVP |
| 21, Control | 28 | RSVP |
| 43, Exp. | 34 | C |
| 44, Exp. | 34 | C |
| 49, Control | 34 | C |
| 50, Control | 29 | C |

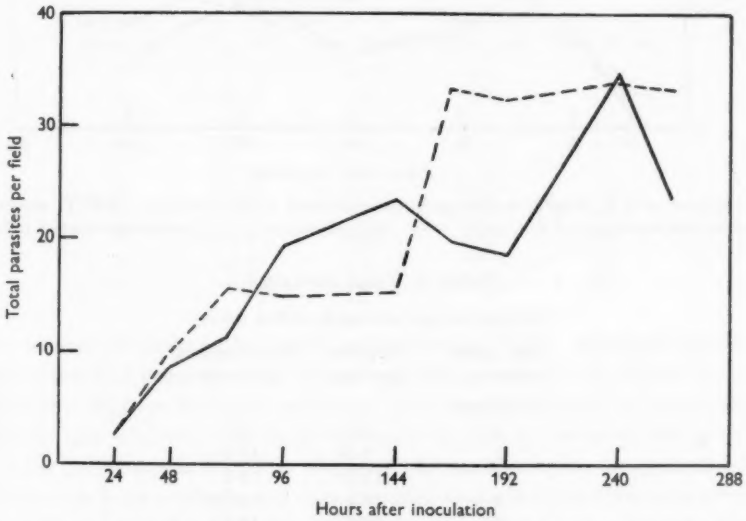


Fig. 3. Course of a *P. berghei* infection in mice treated with cortisone ('C' strain). All points are averages of five mice. ——, control mice; - - - -, cortisone-treated mice.

In Findlay & Howard's work the numbers of parasitized red cells only were counted. Multiple infections of the red cells are very common in *P. berghei* infections. This may be responsible for the rupture of many cells and liberation of parasites in the course of preparing and staining a blood film. A better picture of the effect of cortisone can therefore be obtained by counting the total numbers of parasites per field, including the multiple infections and the parasites liberated from ruptured cells. Such counts are shown in Table 2 and Figs. 3 and 4. There was no consistent difference in the results obtained from the experimental or the control animals, and particularly no evidence was found of excessive multiplication of the parasites in the cortisone-treated animals. Parasite counts in individual animals were more variable in the treated series.

Several authors have suggested that the results previously obtained with cortisone arise from the inhibition of the cellular response to infection. In an attempt to explain the contradictory results that have been obtained it was found necessary

to determine whether cortisone exerted any effect, inhibitory or otherwise, on the multiplication of the parasite in the peripheral blood. For this purpose a count was made of the numbers of schizonts present in each microscopic field, and the

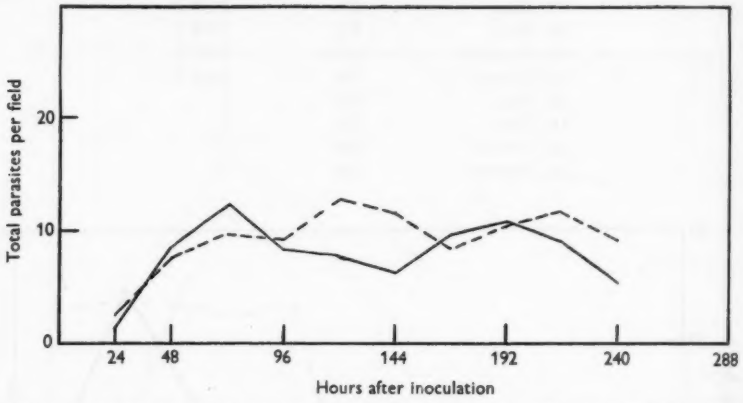


Fig. 4. Course of a *P. berghei* infection in mice treated with cortisone ('RSVP' strain). All points are averages of five mice. ——, control mice; - - - -, cortisone-treated mice.

Table 2. *Total parasites*

(All figures are averages of five mice.)

| Day after infection | Control per field | Experimental per field |
|---|---|---|
| 'C' strain | | |
| 1 | 2·7 | 2·7 |
| 2 | 8·38 | 10·0 |
| 3 | 11·34 | 15·9 |
| 4 | 19·4 | 14·96 |
| 6 | 23·3 | 15·3 |
| 7 | 19·8 | 33·2 |
| 8 | 18·6 | 32·4 |
| 10 | 34·6 | 34·0 |
| 11 | 23·6 | 33·3 |
| 'RSVP' strain | | |
| 1 | 2·16 | 3·7 |
| 2 | 8·56 | 7·56 |
| 3 | 12·28 | 9·8 |
| 4 | 8·28 | 9·12 |
| 5 | 7·6 | 12·8 |
| 6 | 6·16 | 11·4 |
| 7 | 9·6 | 8·25 |
| 8 | 11·76 | 11·64 |
| 9 | 9·04 | 11·92 |
| 10 | 5·4 | 9·12 |

numbers of merozoites in each schizont, throughout the course of the infection in some animals selected at random. Little difference was found between control and experimental animals; although schizonts were more frequent in the experimental ones there was little difference in the numbers of merozoites produced.

RESULTS OF EXPERIMENTS CARRIED OUT ON HAMSTERS

In view of the results obtained by Shwartzman & Fisher similar experiments were performed on young hamsters weighing about 25 g. Each experimental animal and its control came from the same group of hamsters, and were of the same age and weight. Cortisone, 5 mg., was given 3 times at 2-day intervals, beginning 2 days before inoculation with infected blood. Controls received saline as before. Blood

Table 3. *Typical specimen animal, infected with the 'RSVP' strain*

(Mero., merozoites; Schiz., schizonts.)

| Day after infection | Experimental | | Control | |
|---|---|---|---|---|
| | Schiz./field | Mero./Schiz. | Schiz./field | Mero./Schiz. |
| 1 | 1/18·3 | 4·35 | 1/50 | 4·0 |
| 2 | 1/8·1 | 4·3 | 1/27 | 4·0 |
| 3 | 1/4·1 | 4·0 | 1/7·5 | 4·3 |
| 4 | 1/5·4 | 3·9 | 1/4·0 | 4·3 |
| 5 | 1/1·2 | 4·4 | 1/3·3 | 5·3 |
| 6 | 1/2·9 | 4·0 | 1/25 | 5·0 |
| 7 | 1/7·8 | 3·4 | 1/23 | 4·0 |
| 8 | 1/1·1 | 4·85 | 1/25 | 4·0 |
| 9 | 1/3·3 | 3·6 | 1/3·0 | 4·2 |
| 10 | 1·5/1·0 | 4·5 | 1/7·5 | 4·2 |

films were taken, stained with Giemsa, and examined daily. Although the doses of both cortisone and parasites (8 % of the red cells present in the inoculated blood were parasitized) were high, the cortisone instead of producing the rapid increase in infection had a strong inhibitory influence on the parasites in the peripheral blood, most noticeable in the early stages of the parasitaemia. Later in the course of the infection both controls and experimental animals often showed a very high degree of anaemia and parasitaemia. The results of these experiments are summarized in Table 4.

Table 4

| Hamster group | Day after infection | Experimental per field | Control per field |
|---|---|---|---|
| HA | 1 | 0/50 | 0·25 |
| | 2 | 0·06 young rings | 0·6 nearly mature |
| | 3 | 0·1 | 4·0 |
| I | 1 | 0/50 | 0/50 |
| | 2 | 0/50 | 0·2 |
| | 3 | 0/50 | 0·9 |
| | 4 | 0·1 | 5·2 young |
| | 5 | 0·3 | 7·1 all stages |
| II | 1 | 0·1 | 0·15 |
| | 2 | 0·1 | 3·3 |
| | 3 | 0·2 | 6·0 |
| III | 2 | 0·05 | 1·0 |
| | 3 | 0·1 | 7·0 |
| | 4 | 2·0 | 9·0 |

The numbers of merozoites per schizont of a typical experimental hamster and its control are shown in Table 5.

Table 5. *'RSVP' strain*

| Day after infection | | Merozoites/schizont: Range | Merozoites/schizont: Average | Schizonts per field |
|---|---|---|---|---|
| 3 | Experimental | 4–10 | 6·6 | 0·4 |
| | Control | 8–16 | 9·4 | 2·3 |
| 4 | Experimental | 4–6 | 5·0 | 0·3 |
| | Control | 6–12 | 9·5 | 1·5 |

The results obtained from these hamsters appear to indicate a depressive action of cortisone on the multiplication of the parasite, at an early stage in the infection. The average number of merozoites per schizont differs noticeably in the experimental and control animals, in contrast with the results obtained from mice infected with the same strain in which it was very similar and remained constant throughout the infection.

## DISCUSSION

Cortisone is said to inhibit the cellular response to plasmodial infection, preventing the proliferation of macrophages, and so permitting a great multiplication of the parasites. The merozoites on being liberated from schizonts are not removed by macrophages and are free to infect new cells, with a consequent rapid increase in the parasitaemia.

The results published by previous workers have not been supported in the present work. It has been found that cortisone does not cause any rapid increase of parasites in the peripheral blood of mice or hamsters, and in hamsters its administration has a marked inhibitory effect.

In the absence of further investigation into the precise nature of the action of cortisone upon the reticulo-endothelial system it must be assumed that in animals treated with cortisone macrophages are fewer in number, and phagocytosis is less active than in animals not so treated. The presence of some other humoral agent in the peripheral blood is therefore postulated, which has the effect of restricting the increase of parasites despite the influence of cortisone on the cellular response.

## SUMMARY

Contrary to results previously published, cortisone did not cause a rapid increase of parasitaemia in mice or hamsters infected with *P. berghei*, and in some cases it had an inhibitory effect. The existence of a humoral agent restricting the multiplication of the parasites in the peripheral blood was suggested.

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# A NEW SPECIES OF *OTOBOTHRIUM* (CESTODA, TRYPANORHYNCHA) FROM AUSTRALIAN FISHES

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(With 6 Figures in the Text)

## 1. INTRODUCTION

In April 1953, several specimens of the common commercial mullet, *Mugil cephalus* L., heavily infested with cysts in the body cavity, were received. In May, June and July 1953, several specimens of the estuarine catfish, *Netuma australis* (Gunther) (Family Ariidae), similarly infected, came to hand. All specimens were taken from the lower reaches of the Brisbane River, Brisbane.

The cysts contained larval trypanorhynch cestodes of the genus *Otobothrium* Linton, 1890, subgenus *Otobothrium* (see Dollfus, 1942). The genus has not been recorded previously from Australia, and the species, which appears to be new, is described hereunder as *Otobothrium* (*Otobothrium*) *mugilis* n.sp., the specific name indicating the common host.

This parasite also appears to be the first recorded from the mullet in Australian waters.

In a search for the adult tapeworm of this species, four species of sharks have been examined, viz. the brown-spotted catshark, *Hemiscyllium punctatum* Muller & Henle, 1838; the weasel-shark, *Hemigaleus microstoma* Bleeker, 1852, the gummy-shark, *Mustelus antarcticus* Gunther, 1870, and the dogshark, *Scoliodon longmani* Ogilby, 1912. The search has so far proved fruitless: the ensuing description is based, therefore, on larval material only.

## 2. TECHNICAL

Fixation was in warm Bouin's; whole material was stained with alum carmine, cleared in methyl benzoate and mounted in Gurr's medium. 7$\mu$ paraffin sections were stained with Mallory's Triple stain and mounted in balsam. Evagination of proboscides was accomplished prior to fixation by gentle squeezing of the bulbs. All figures are from camera lucida outlines.

## 3. DESCRIPTION OF *OTOBOTHRIUM* (*O.*) *MUGILIS*, n.sp.

### (*a*) *Cysts*

In *Mugil*, the cysts were distributed sporadically in the gut mesentery (along with gasterostome trematode larvae) and *on* the peritoneum. In *Netuma* they were confined to the body wall, lying between the peritoneum and musculature. They ranged from 1 to 3 mm. in diameter, the largest resembling rice grains. A few much larger 'cysts' (6 mm. in diameter) were aggregations of up to five cysts of normal size.

The cysts consist of three distinct and separable parts. A layer of mesentery composed of host tissue overlies a thin, acellular, transparent layer which in turn covers the plerocercus blastocyst proper. Though the latter appeared completely closed, serial sectioning gave evidence of the scolex or holdfast region having sunk into the cyst, the aperture so formed closing subsequently. There was no invagination *sensu stricto*.

### (*b*) *Scolex*

The scolex lies eccentrically within the cyst (Fig. 1) and is easily removed.

Table 1 shows the dimensions of its parts (terminology from Dollfus (1942); measurements in micra; based on ten fixed, mounted specimens).

Table. 1

| Part | Minimum | Maximum | Mean |
|---|---|---|---|
| Total length (including appendix) | 723 | 970 | 843 |
| Pars bothridialis | 238 | 270 | 256 |
| Pars vaginalis | 400 | 539 | 480 |
| Pars bulbosa | 200 | 231 | 214 |
| Pars postbulbosa | 15 | 46 | 23 |
| Velum | 46 | 169 | 114 |
| Appendix | 231 | 262 | 241 |
| Greatest breadth | 354 | 462 | 388 |

The bothridia are two in number, patelliform, and lie one dorsally, one ventrally, inclined together at a slight angle anteriorly. Their lateral and posterior margins are free and thickened as a bourrelet; the posterior margins do not bear a notch. Symmetrically, lying postero-laterally in each bothridium are two retractile organs (ciliated pits) whose aperture opens slightly inwards (Figs. 2, 3). The bothridia are 0·25 mm. long and slightly less wide.

### (*c*) *Proboscis apparatus*

The scolex bears four proboscides or tentacles, each consisting of a muscular bulb, retractor muscle, proboscis sheath and evaginable proboscis.

The bulbs, which lie posteriorly, are 0·21 mm. long and 0·11 mm. broad. Their walls are made up of several layers of muscle fibres set alternately in a criss-cross pattern (Fig. 2); the lumen of each bulb is only one-third of the overall diameter.

The retractor muscles of the proboscides are inserted near to the entrance of the sheath.

The proboscis sheaths are clear, tubular structures passing from the anterior extremity of each bulb to open, one pair dorsally, one pair ventrally, near the anterior border of the corresponding bothridium. Each sheath, throughout its length, is coiled spirally about one of the four longitudinal muscle bundles which traverse the scolex (Figs. 2, 3 *m*).

The evaginable portion of the proboscis, when fully invaginated, reaches to below half of the length of the sheath. The fully evaginated proboscis has a short, swollen, basal region passing to a narrower, long, distal region. The oncotaxy of the two regions differs. The fully evaginated proboscis measures 0·86 mm., including the basal region (0·12 mm.).

### (*d*) *Proboscis armature*

The basal portion of each proboscis (Fig. 5) bears three types of hooks. (i) Two rows, basal length about $8\mu$, lie along the bothridial and antibothridial surfaces. (ii) The internal face is beset with several rows of smaller hooks, basal length less

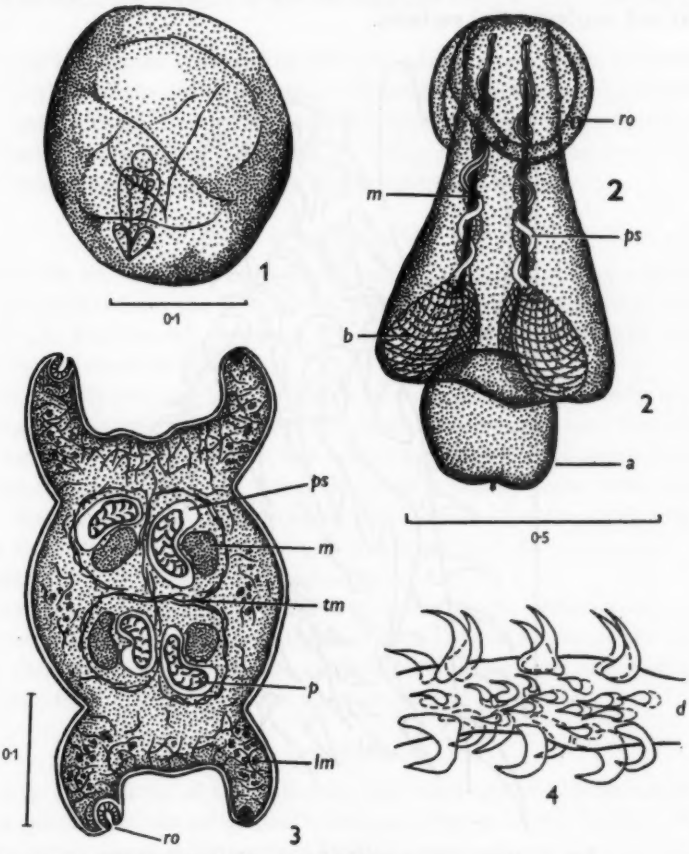


Fig. 1. Entire blastocyst from *Mugil cephalus*.
Fig. 2. Dorso-ventral view of isolated scolex.
Fig. 3. Transverse section scolex, bothridial region, through retractile organs.
Fig. 4. Hook arrangement of proboscis, mid region, viewed from external surface.
(Scale shown in mm.; Fig. 4 drawn to same scale as Figs. 5 and 6.)

*Key to lettering of figures.* *a*, appendix; *b*, bulb at base of proboscis sheath; *d*, distal end of proboscis; *lm*, longitudinal musculature of bothridia; *m*, longitudinal muscle bundle of scolex; *p*, proboscis in sheath; *ps*, proboscis sheath; *ro*, retractile organ; *tm*, transverse musculature.

than $5\mu$, which give way distally to larger hooks on the proboscis. (iii) On the internal face of each basal portion is one very large hook (Fig. 6), facing proximally, with a basal length of about $45\mu$.

The armature of the distal portion of the proboscis (Fig. 4) is typical of the family Otobothriidae Dollfus, 1942, i.e. atypical heteroacanthous (Dollfus). Bands of larger hooks (*'crochets principaux'* of Dollfus (1942)), basal length about 10$\mu$, arise on the internal face and pass obliquely and distally across the bothridial and antibothridial faces, but do not trespass on to the external face. The latter is beset with several rows of smaller hooks, basal length 3–4$\mu$, which extend on to the bothridial and antibothridial surfaces.

Fig. 5. Hook arrangement of proboscis, basal portion.
Fig. 6. Large hook from Fig. 5.
(Scale shown in mm.; Figs. 5 and 6 drawn to same scale.)

### (e) *Musculature*

In most respects, the musculature of both the bothridia and scolex follows a typical pattern. The longitudinal musculature, however, presents certain features which have not been recorded in the genus. At least three distinct arrangements of longitudinal muscle can be distinguished. In the bothridia, numerous fibres are scattered through the tissues, with aggregations prominent laterally (Fig. 3, *lm*). In the scolex below the level of the bothridia, a delicate layer of longitudinal fibres

is found superficially, below the basement membrane. The third group of longitudinal fibres runs in four prominent bundles through the scolex (Fig. 2, *m*), each forming a core around which a proboscis sheath is coiled. In whole mounts, these bundles appeared to be equivalent to the posterior longitudinal extrinsic muscles as figured by Rees (1944, fig. 1) for *Grillotia acanthoscolex* (Family Lacistorhynchidae). The latter are present in two bundles and are inserted anteriorly into the bothridia.

Sectioning has shown that, in the present species, the bundles originate in the posterior portion of the scolex and pass unbranched to its anterior end where they converge into a small knot of fibres. They appear to have no relation with the longitudinal muscles of the bothridia. In living specimens, the four bundles can contract independently and give the scolex extreme flexibility.

## 4. BIONOMICS

Mullet for sale on the local market are caught in Moreton Bay and along the adjoining coastline from the New South Wales border to about 200 miles north of Brisbane. The Fish Board, Brisbane, reports that infections of cysts have never been noticed in these mullet.

Catches, usually private, are made on a lesser scale in the Brisbane River, from an effectually estuarine habitat. Mr W. Green of Brisbane, who first noticed an infected mullet from this locality, has kindly supplied the following information. He has taken mullet from the Brisbane River annually since 1935; until April 1953, he has never noticed an infected fish. From this time until the mullet migrated from the river in May 1953, he has observed a 75 % infection. Further, he has found no 'first-class' fish this season.

The percentage infection of *Netuma* appears to be considerably lower: of thirteen fish examined, five were infected and only one heavily so. It is conceivable, therefore, that the present epidemic is localized and of recent origin; of the fish examined, the mullet is the most frequently parasitized.

## 5. AFFINITIES

The relative dimensions of the bulbs and the insertion of the retractor muscles place this species into the subgenus *Otobothrium* segregated by Dollfus (1942). The size of the scolex places it further into Dollfus's subgroup A—'Scolex très petit'—which includes *O.* (*O.*) *crenacolle* Linton, 1890, *curtum* Linton, 1909, *carcharidis* (Shipley & Hornell, 1906), *cysticum* (Mayer, 1842), *balli* (Southwell, 1929) and *pronosum* (Stossich, 1900).

*O.* (*O.*) *mugilis* differs markedly in size from these species except *crenacolle* from which it differs in having relatively larger bulbs and undivided bothridia. The large hook on the basal portion of the proboscis is found also in *curtum*, but the latter has bothridia which overhang the bulbs and the proboscis sheaths are nearly straight.

Host-lists for adults and/or larval stages of the above species are given by Dollfus (1942). The larvae have been recorded from a variety of teleost fishes and that of *O.* (*O.*) *cysticum* from *Chelone mydas* L. as well. None, so far as can be determined, have been recorded from the hosts of the present species.

### 6. DIAGNOSTIC FEATURES

Blastocyst small, 1–3 mm. diameter, scolex 0·8 mm. long, 0·4 mm. wide; bothridia patelliform with bourrelet, 0·25 mm. long; proboscis with enlarged basal portion bearing three types of hooks, one being single, large and characteristic; bulbs 0·21 mm. long, 0·11 mm. diameter; retractor muscles inserted near entrance of sheath.

### 7. TYPE MATERIAL

The holotype (mounted slide) of *Otobothrium* (*O.*) *mugilis* n.sp. has been deposited with the Queensland Museum. Paratypes have been sent to the Muséum nationale d'Histoire naturelle, Paris, and to the U.S. National Museum, Washington, D.C.; further paratypes are retained in the University of Queensland.

The author is indebted to Mr T. C. Marshall, Queensland Government Ichthyologist, for referring the infected mullet to him and for kindly identifying the sharks examined. Mr W. Green of Brisbane provided several specimens of *Mugil* for examination and the records used in §4, above. Prof. W. Stephenson of the University of Queensland procured several specimens of *Netuma* and offered advice in the preparation of this paper. Acknowledgement is made to the Commonwealth Research Grant, University of Queensland, for financial assistance.

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# ELECTRON AND LIGHT MICROSCOPE STUDIES OF THE DEVELOPMENT OF THE VIRUS RODS OF INSECT POLYHEDROSES

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(With Plates VII–XII)

## INTRODUCTION

In 1915 Glaser demonstrated for the first time that a nuclear polyhedrosis of a lepidopterous larva (*Lymantria dispar*), was induced by an invisible filterable virus isolated from the blood of diseased larvae. Nuclear polyhedroses of the larvae of over a 100 insect species are now known, and those of them which have been investigated have been shown to be due to viruses. In these polyhedroses the nuclei of susceptible cells, hypodermis, tracheae, fat and blood cells, etc., enlarge and become packed with infectious crystalline inclusion bodies, the polyhedra, from which the diseases get their name. The pupae and imagos of these insects are not generally susceptible to polyhedral virus diseases, and so far no nuclear polyhedrosis of imaginal tissues in the larvae has been reported. This has led to the suggestion that imaginal tissues are not susceptible to polyhedral virus diseases. This however is not the case, and we have found typical nuclear polyhedroses of imaginal tissues in the larvae of several species, e.g. nuclear polyhedroses of the imaginal wing and limb buds in *Abraxas grossulariata* and *Tineola bisselliella*, of the gonad cells in *Phlogophora meticulosa*, and a cytoplasmic polyhedrosis of the basal replacement cells of the gut of *P. meticulosa*. It may also be stated that we have found a virus, or virus complex, in *Tineola bisselliella*, the clothes-moth larva, which causes a well-developed and typical nuclear polyhedrosis of all the following tissues, hypodermis, fat body, silk gland, Malpighian tubules, nerve ganglion cells, imaginal buds, fore-gut and mid-gut.

We have examined the intranuclear changes of infected cells of many species and have found marked differences between the species (Xeros, 1953*a*, *b*). However, the main features of the sequence of intranuclear changes are identical. The nucleus of an infected cell grows enormously, and most of the chromatic material, which is now greater in bulk than that of normal nuclei, clumps together to form an off-central oval chromatic mass. The remainder becomes attached to the inner surface of the nuclear membrane, to which the central chromatic mass also appears to be attached. Between the two lots of chromatic material a clear ring zone develops, refractile to staining. Later, after the transformation of the central chromatic mass to a network, polyhedra form in the ring zone and grow from about 0·2 to 0·3$\mu$ to 1$\mu$ or more in diameter, and may even reach 10$\mu$.

In 1943, Glaser and Stanley, on the basis of comparative biochemical studies of free virus, obtained from centrifuged blood of diseased larvae, and of polyhedra, concluded that though virus was occluded in the polyhedra, no great concentration was to be found within them. The polyhedra dissolve in weak sodium carbonate, and Bergold (1947) demonstrated by electron microscopy the virus rods enclosed in the polyhedral membranes after alkali dissolution of the protein body of the polyhedra. The polyhedral protein has been found to be about 80 % of the weight of the polyhedra, though this is only a statistical figure. The virus rods are composed of desoxy-ribose nucleoprotein.

In order to study the mode of development of the virus rods of these polyhedroses, it is necessary to identify the site of formation of the virus. The only studies which have so far been made in this direction have been made with the light microscope on species in which the virus is now known to exist predominately in the form of bundles of several virus rods. As each rod may be about 300 by 40$\mu$ in size, the larger bundles at any rate can be seen with the light microscope. The most carefully studied of these species have been *Lymantria dispar* and *L. monacha*. Recently, Xeros (1953*b*) developed a method for staining the virus bundles within polyhedra, and was as a result able to identify them in the nuclei of *L. dispar* and *L. monacha* prior to the formation of polyhedra.

The staining method involves pretreatment of Carnoy-fixed sections in 1N-HCl at 60° C. for 10 min., staining in Giemsa solution for 20 hr. or more, and differentiating in 95 % alcohol for 5–20 min. The virus bundles, both within and without the polyhedra, are in this way stained a deep blue or reddish purple colour and can be readily identified. The polyhedral protein stains a weak pink colour. The origin and fate of the virus bundles in *L. dispar* and *L. monacha* can be traced in sections of diseased larvae treated in the above manner. It is fortunate that in the formation of the central chromatic and peripheral chromatic masses the large chromatin bodies of the normal nuclei fuse to leave a clear ring zone. After the formation of the chromatic mass and ring zone free virus bundles make their first appearance, alongside the central chromatic mass. In the early periods of free virus bundle formation their density is greatest alongside the central mass which transforms to a network as free virus bundle formation proceeds. Larger chromatic strands may also detach from the chromatic mass as it tends to fray in parts, and break down to virus bundles as the disease progresses. A pale blue-staining residue is left behind as this 'breakdown' occurs, suggesting that the formed bundles slip away from a surrounding matrix. Bodies similar to the free virus bundles become visible within the chromatic mass, and these appear to detach progressively from it leaving a chromatic network whose strands are less chromatophilic and less irregular in staining capacity. Both the bromophenol blue (protein) and the Feulgen (DNA) staining capacity of the chromatic body decreases as these changes continue. A similar breakdown of peripheral chromatin to free virus bundles also occurs during this time. Thus prior to the formation of any polyhedra in the ring zone, large numbers of virus bundles are accumulated there, and as the polyhedra grow the former become embedded in them and can be shown there by the method described above. Indeed in these two species the polyhedra often originate by

deposition of polyhedral protein around aggregations of large numbers of virus bundles. The virus bundles adhering to the surface of the growing polyhedra, or partially embedded in them, and those still free can be readily recognized. As the growing polyhedra begin to pack the individual nuclei, more and more virus bundles are found inside them and fewer and fewer outside them. Finally appreciable numbers of free virus bundles occur only around the central chromatic residue. The correspondence between the amount of staining material inside the polyhedra of the polyhedron-filled nucleus and in the ring zone just prior to the formation of polyhedra is very close. Since from our electron microscope studies we know that the virus in the polyhedra of these species is predominately in the form of virus bundles of two or three rods per bundle, and there even appears to be a secondary association of two or more bundles into groups, it appears justifiable to conclude that the site of virus formation in these species is in the chromatic material of the diseased nuclei, and predominately in the central chromatic mass. The data of the light microscope studies seem consistent with a further suggestion that virus formation may occur by differentiation of anomalous nucleoprotein material of the chromatic masses rather than by division and subsequent growth of spherical virus organisms of about 40$\mu$ in diameter.

It has already been mentioned that in 1915 Glaser isolated the virus of the nuclear polyhedrosis of *L. dispar*. On the basis of this fact he wrongly concluded that the bodies forming in diseased nuclei and accumulating in the ring zone, identified above as virus bundles, were not causal agents of the disease but non-infectious by-products of it. However, on the basis of his cytological observations, he concluded that these bodies, visible also in Giemsa-stained material not pretreated with HCl, were formed in the central chromatic mass, and from there entered the ring zone. Komarek & Breindl (1923), though they believed the bodies to be micrococcus-like virus organisms and causative agents of the disease, likewise concluded that they multiplied in the chromatic mass in *L. monacha*. The latter workers also considered that these bodies became embedded in the polyhedra, somewhat similar unstained bodies being visible in formed polyhedra. Xeros's (1953*a*, *b*) observations agree with the earlier workers in placing the origin of the virus bundles in the chromatic masses of the diseased nuclei.

In infected nuclei of *Bombyx mori* the course of intranuclear events is somewhat different. In this species visible virus bundles are not often found in the ring zone. Electron microscopy of the viral contents of the polyhedra confirms that the virus rods in this species are almost exclusively in the form of single virus rods. Moreover, immediately prior to the formation of polyhedra, considerable and increasing amounts of protein material, staining a pale pink with Giemsa and a medium blue with bromophenol blue, is found precipitated in the ring zone. Later relatively few and very large polyhedra form in the infected nuclei from this protein and leave the nuclear sap free of visible protein deposit. In this species, then, large amounts of polyhedral protein are to be found in the ring zone even before polyhedral formation. The nuclear polyhedra of *B. mori* appear to be more permeable to dye than the polyhedra of other species, and even without prior HCl treatment the virus contents of many of them may stain a pale blue after standard treatment with

Giemsa or bromophenol blue stains, or in heat-dried smears stained with stock solution of Giemsa stain. In these circumstances, however, the protein of the polyhedra does not retain the stain.

## MATERIALS AND METHODS

The main purpose of this paper is to report the findings of electron microscope studies of sections of *B. mori* infected with its own nuclear polyhedrosis. The strain of polyhedral virus used in these studies was one kindly supplied by Dr Bergold. Third or fourth instar larvae, two-thirds of the way through the disease, were used. In fixing, the larvae were first injected with a 2% osmic acid solution buffered at pH 7 with phosphate buffer, and immersed in the same fixative. After some minutes the larvae were cut into a number of pieces and left in the fixative for 2 hr. They were then washed, dehydrated by very gradual passage through the alcohols, embedded in methacrylate and thinly sectioned, using a specially adapted rotary microtome. The sections mounted on formvar-covered grids were treated with amyl acetate to remove the plastic and shadowed lightly with palladium before being examined in a Metropolitan Vickers electron microscope using 75 kV. Micrographs were taken mostly of fat tissue nuclei, though these showed no difference from the pictures of hypodermis and tracheal cell nuclei. Acknowledgments are due to Miss Sheila Vernon Smith who took the photographs.

## RESULTS

Figs. 3–13, in Pls. VII to XII, are electron micrographs of typical diseased nuclei of fat cells of *B. mori*. They are arranged in order to show the course of intranuclear changes during polyhedrosis. The fixation of this material is strongly reminiscent of alcohol precipitation rather than osmic acid fixation, and is probably due to inadequate prior fixation in the osmic acid. The precipitation also makes it virtually impossible to observe the fine structure of the chromatic masses. Nevertheless, the micrographs show many important features. The enormous enlargement of the nuclei, followed by the formation of the central chromatic mass, the adherence of the remainder of the chromatin to the nuclear membrane, and the formation of the clear ring zone are readily visible. Accumulation of free virus rods in the ring zone, followed by the accumulation of polyhedral protein in the ring zone where it is precipitated around the virus particles by fixation, can also be seen. Finally, the polyhedra, within which the virus rods become embedded, form from this polyhedral protein, leaving the nuclear sap again free of insoluble contents.

The earliest stage at which free virus rods have been found is shown in (Pl. IX, fig. 7. At this stage the central chromatic mass has only recently been formed. At no earlier stage has a virus rod ever been found, nor has one ever been detected in the cytoplasm. The virus rods in these earliest stages of virus rod formation are found alongside the chromatic masses. Pl. X, figs. 8, 9, show later stages of virus formation, the ring zone becoming increasingly filled with virus rods. In these nuclei too numerous virus rods appear to be protruding from the central chromatic masses, others seem to be protruding from the peripheral chromatin. Pl. IX, fig. 7,

gives the appearance of several bundles of virus rods being protruded from the central chromatic mass and from peripheral chromatin bodies into the ring zone where the individual rods separate from one another. In Pl. VIII, fig. 5, a long virus body 500$\mu$ in length can be seen protruding from peripheral chromatin. Similar rods almost twice the normal length of the virus rods are present in other sections, in some cases giving the appearance of breaking across to form smaller sized rods.

The distribution of the virus rods in the infected nuclei show several features, which suggest that the virus rods form in the central and peripheral chromatic material, predominately in the latter. A great proportion of the virus rods, particularly while they are still few in number, are found partially embedded in the chromatic material, presumably in the process of slipping out of these masses. A gradient of concentration of virus particles, highest nearest the masses and decreasing away from them, is definitely suggested by the pictures. The alcohol-type precipitation of the central chromatic masses, however, prevents a clear understanding of the way in which such a slipping out might occur, if indeed the virus rods do not form simply as the result of a cylindrical protruding of material at the surface of the masses. In the nuclei with osmic acid-type fixation (Pl. XII, figs. 12, 13), and in which the polyhedral protein is fixed *in situ* and not coarsely precipitated, it can be further seen that the central chromatic mass has a very marked, fine fibrillar structure in addition to a smoothly deposited material. A coarse fibrillar structure alone is also visible in finely sectioned alcohol-type fixed-nuclei. A fibrillar structure has not, however, been observed in the chromatin bodies prior to the formation of the central chromatic mass, either in alcohol or osmic type-fixed nuclei. The fibrils of the chromatic mass are found to be of varying thickness up to the diameter of virus rods, from which some of the fibrils seem indistinguishable (Pl. XII, fig. 12). The thickness of normal and half length rods isolated from polyhedra appears, however, to be much the same. It is also known that the Feulgen and Giemsa staining capacities of the chromatic masses fall off after their great initial increase, and the chromatic mass is seen to transform into a marked network in Carnoy-fixed material. Together these facts suggest that the virus rods may arise in the chromatic masses by differentiation into fibrils of anomalous pre-virus materials which accumulate in the chromatic masses following re-direction of the cell metabolism by virus infection. The possibility that long fibrils may also differentiate and later break down to normal or smaller sized virus rods is strongly suggested. It seems that the virus rods are then freed or protruded into the ring zone where they accumulate.

From preliminary light microscope studies on larvae infected with extremely heavy doses of virus and killed at 2-day intervals thereafter, it appears that the swelling of the nuclei and the process of clumping of the chromatin material is the longest single period in the life of the infected nucleus, the period of polyhedron formation being the shortest. This situation along with the data above suggests a long preliminary latent period following infection, during which no genetically continuous virus rod or sphere is present, and supports a non-organismal view of the nature of these viruses.

The situation found in tracheal and hypodermal cells is similar to that in the fat cells. It may be remarked that the free virus rods in the nuclear ring zones in these sections are readily visible on the electron microscope fluorescent screen.

It is of interest to note that the free virus rods (Pl. IX, fig. 7, Pl. X, fig. 8) do not have a surrounding capsule. Yet the rods dissolved out of polyhedra by weak sodium carbonate solutions do have very definite and well formed enclosing capsules (Pl. VII, fig. 2). It seems clear that the perivirus capsule material must be aquired by the virus rod in the ring zone, probably as it becomes immersed in polyhedra protein. In Pl. VIII, figs. 5, 6 and Pl. IX, fig. 7, another curious feature in the morphology of many of the virus rods may also be noted. Often, at one end, sometimes at both ends, of a virus rod, a small spherical body about the same diameter as the width of the virus rod may be seen These spherical bodies are also found in the same situation in some virus rods dissolved out of polyhedra. It is not clear whether these are formed by fragmentation of rods. On prolonged alkali treatment of polyhedra, the freed virus rods tend to dissolve from one or both ends leaving behind a collapsed intimate membrane which had been closely applied to them. In Pl. VIII, fig. 5, there are several cases where two rods appear to be enclosed one behind the other in the same continuous membrane. In addition, in Pl. IX, fig. 7, there can be seen a structure resembling an intimate membrane but in which only a few spheres are present. Bergold (1953) figures virus rods extracted from polyhedra and treated with trypsin. These micrographs show virus rods as a series of spherical bodies or bands within the intimate membranes. Such a 'breakdown' also occurs with HCl treatment. The significance of this periodicity within virus rods is not clear.

Apart from the view of the nature of virus multiplication outlined above, one must consider the possibility that the virus either multiplies or first forms in the ring zone from minute spherical germs which later grow into elongated rods (Bergold, 1950). Several features of the micrographs are worth mentioning in this connexion. First very few spherical bodies which might be virus 'germs' have been found in the nuclei before the formation of the central chromatic masses and the appearance of well-differentiated rods in the ring zone. Even after the appearance of free virus rods few spherical bodies are found in the ring zone and some of these may be small pieces of virus rods alined at right angles to the plane of sectioning. Again, large numbers of similar bodies are to be found in the cytoplasm, and there is no evidence suggesting that these are virus bodies. The fact that no appreciable number of free virus rods, one-third to two-thirds normal length appear in the ring zone prior to the appearance of normal sized rods, or even after their appearance, also seems to us strong evidence against the view that the virus rod grows from a minute sphere free in the ring zone. Nevertheless, a number of small spherical bodies, some even giving the appearance of having divided, are present in the ring zone, and though we are not at the moment inclined to consider these bodies as spherical multiplication stages of the virus, it is clear that more work is needed to determine their significance. To what extent such small spheres may have been removed by the amyl acetate used to remove the plastic embedding medium is not yet known.

It has already been stated that though in *Lymantria monacha* the virus rods are mostly in the form of bundles of several rods, in *Bombyx mori* they are practically all single rods. However, secondary associations of numbers of virus rods, each with their surrounding capsule, may occur in both species. The large highly refractile bodies which can be seen in the polyhedra of *B. mori* with the light microscope even without any staining of them are undoubtedly such associations of virus rods (Pl. VII, fig. 1). Some of the V forms described by Bergold (1950) as stages in the development of virus rods are also undoubtedly associations of two virus bundles of half length rods. In the bundles of 2, 3, or more virus rods in *Lymantria monacha*, each rod has not got its own separate capsule, but the virus rods are closely applied and have one capsule only surrounding the whole bundle. In Pl. IX, fig. 7, likewise, a number of virus rods closely connected and without any capsules are seen protruding from the chromatic masses. It seems correct to conclude that large numbers of rods may form together and become secondarily separated or become surrounded by capsule protein before they have separated. In a quantitative study of normal length virus rods in *L. monacha* we have found the ratios of the number of bundles of 1, 2, 3, or 4 virus rods to be 37:85:33:9. The presence of so many bundles with 3 virus rods would be difficult to understand if the number of rods in a bundle depended on the number of divisions of a spherical virus germ within an outer membrane.

Little evidence has as yet been found as to the site of formation of the polyhedral protein. Though in *Bombyx mori* large quantities of it accumulate in the nuclear ring zone prior to the formation of polyhedra, this does not appear to be the case in other species, e.g. *Lymantria monacha*, *L. dispar*, *Abraxas grossulariata*, *Phlogophora meticulosa*. In these species it seems as if the entry into or formation in the ring zone of polyhedral protein coincides with free virus bundle formation (*Abraxas grossulariata*, *Phlogophora meticulosa*), or occurs after an almost complete complement of free virus bundles accumulates in the ring zone (*Lymantria monacha*, *L. dispar*). However in all these four species, as distinct from *Bombyx mori*, the polyhedral protein appears to deposit around virus and form polyhedra as soon as any quantity of it at all is found in the ring zone. The site of formation of the polyhedra does not add much, for whilst in *Abraxas grossulariata* the first polyhedra form at the periphery of the nucleus, in *Phlogophora meticulosa* they form both there and around the central chromatic masses, whilst in *Lymantria dispar*, *L. monacha*, and *Bombyx mori* they form more or less centrally in the ring zone. In the nuclear polyhedroses, however, the cytoplasm, particularly of the hypodermal and tracheal cells, greatly enlarges as does its ribose nucleic acid. It is conceivable that the polyhedral protein is formed in the cytoplasm and from there enters the ring zone. The precipitated polyhedral protein found in the ring zone prior to polyhedral formation in *B. mori* does not stain any more intensely than the cytoplasm, though the polyhedra stain far more deeply. Moreover, the precipitated polyhedral protein stains with bromophenol blue even without prior HCl treatment, though the protein of the polyhedra do not. The inability of the polyhedra to fix stains without prior HCl treatment is therefore due to their peculiar chemical state, their great staining capacity after HCl treatment probably due to their enormous concentra-

tion of protein, and the staining properties with bromophenol blue seem of little use in determining the site of polyhedral protein formation, if indeed it does not arise *in situ*. However, in both the alcohol and osmic acid type-fixed sections used for electron microscopy (Figs. 3–13 in Pls. VII–XII) the polyhedral protein seems to be most dense at the periphery of the cell nucleus. If the polyhedral protein were issuing from the chromatic mass this would hardly be the case. So, though the bromophenol blue staining capacity of the nuclear mass or early net is very high and is somewhat increased by HCl pre-treatment, this may be due to the formation of virus or pre-virus protein rather than to any formation of polyhedral protein there.

## DISCUSSION

In considering the electron micrographs of the various stages of development of diseased nuclei we have been concerned to discuss those features which seemed to us the most significant. It appears that it can be definitely stated that the virus does not have a genetically continuous existence as a virus rod, that the virus rods develop in intimate connexion with the chromatic masses and that the perivirus capsules found round rods isolated from polyhedra are secondarily deposited around them whilst they are in the ring zone. The evidence also suggests the origin of the virus rods in the chromatic masses, and their differentiation there from anomalous pre-virus material by the formation and gradual thickening of fibrils within these masses. Though more work is necessary unequivocally to establish these conclusions, we consider the evidence sufficiently suggestive to warrant their being drawn and becoming the subject of further research. Bergold (1950), in his studies of the viral contents of polyhedra, concluded that the virus was a genetically continuous organism multiplying by growth and fission, and that eventually each minute spherical 'germ' grew to a rod. Some of his interpretations, e.g. of the relation of certain rods and membranes, and also certain of the V forms, found in his differentially centrifuged preparations, are implausible; his evidence is open to the interpretation of the nature of virus development we have outlined above. This possibility, however, does not appear to have been seriously considered by Bergold (1950, 1952), nor does he supply evidence that the virus multiplies and develops in the ring zone. No evidence is afforded for the view that the virus is a genetically continuous organism.

Earlier we drew attention to the fact that the free virus rods in the sections do not possess perivirus capsule material, which is possessed, however, by the virus rods extracted from polyhedra. The evidence indicated that the capsule was acquired by the free rod whilst immersed in the polyhedral protein. The absence of the capsule is of importance to the interpretation of Bergold's 'spherical' stages which are roughly of a diameter half the length of normal virus rods. We have made a qualitative and quantitative study (unpublished) of the viral contents of polyhedra of several species, in particular of *Lymantria monacha*, and have found that these 'spherical' stages are in reality made up of several short rods surrounded by perivirus capsule material. All sized rods between the longest (360$\mu$) of the normal sized rods and the shortest (120$\mu$) of the half-sized rods were found, and their distribution forms a continuous curve with two maxima. One maximum is

at the normal size (295$\mu$), and the other far less pronounced maximum is at about 145$\mu$, almost half the size of the normal rods. In *Bombyx mori* the two maxima also occur at the same relative sizes. In both species the thickness of the normal and half-sized rods is much the same. The thickness of the fibrils of the central chromatic masses, however, varies from extremely fine to that of normal virus rods. These facts, and the existence of two maxima, with virus rods mainly of normal and half lengths, not merely in one species but in several that we have examined, suggest that these may be the two most probable sizes of differentiated virus material, rather than that the smaller are stages in the development of a virus organism. It must also be stated that in these studies as those of others no great efforts have been made to isolate pure strains and see whether infection with such a strain would also give rise to similar qualitative and quantitative data on the viral contents of polyhedra. We have, however, been able to establish that the whole range of forms and sizes may exist in a single polyhedron, and therefore that they all arose within a single nucleus (*Lymantria monacha*). We have also found polyhedra whose viral contents were almost entirely made up of 'spherical' forms of diameter about half the length of normal rods, with only a few normal-sized rods present. It should be stated that as yet it is not known that these 'spherical' forms are infectious.

Though it is known that virus appears in the blood of diseased larvae very early in a rapidly developing infection, it is not known how it gets there. Our observations suggest that virus gets into the blood stream only after polyhedron formation and the rupture of nuclei bursting with polyhedra. At an early stage in the disease any polyhedra liberated into the blood would be dissolved and liberate their virus contents there. A most satisfactory and rapid staining method for studying the presence or absence of polyhedra in larvae in the early stages of disease has been found to be as follows. Sections of Carnoy-fixed larvae are treated with concentrated HCl for half a minute and stained with hot Altman's aniline acid fuchsin, for several minutes. In this way the clearest differentiation of central chromatic masses and polyhedra from the cytoplasm, etc., can be obtained.

Two views of the nature of viruses have long been held. One regards them as minute genetically continuous organisms, which multiply by division and growth. The other considers the viruses as complex materials inducing an alteration of cell metabolism, one of the end results of this altered metabolism being the production of more virus materials. Also there is no *a priori* reason to suppose all viruses are identical in their general nature and developmental properties. The full clarification of the position of even one virus requires a many-sided approach, and in this paper we have advanced preliminary data suggesting that the viruses of the insect polyhedroses are not organisms but one of the differentiated products of altered cell metabolism.

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## EXPLANATION OF PLATES

(Photograph for Fig. 1 by S. Frey, all others by S. Vernon Smith.
All sections of *B. mori* material.)

### PLATE VII

Fig. 1. Heat-dried smear, unstained, of polyhedra of *B. mori*, showing refractile virus material within polyhedra (oil immersion lens). × 1100.

Fig. 2. Virus rods with perivirus capsules released from polyhedra by treatment with weak sodium carbonate. × 22,400.

Fig. 3. Section of enlarged nucleus of infected cell showing chromatin bodies, before formation of the central chromatic mass. × 3750.

Fig. 4. Section of nucleus showing formation of central chromatic mass, peripheral chromatin and ring zone; no recognizable virus rods present. × 3550.

### PLATE VIII

Fig. 5. Section of nucleus showing early stage in formation of virus rods; these can be seen protruding from the central and peripheral chromatin. Note absence of perivirus capsules. × 13,750.

Fig. 6. Similar to fig. 5, but a slightly later stage, again showing connexion of virus rods with chromatic masses. × 11,250.

### PLATE IX

Fig. 7. Section of nucleus in a later stage showing free virus rods in the ring zone, and bundles of virus rods apparently being protruded from the central chromatic mass. × 22,500.

### PLATE X

Fig. 8. Similar to fig. 7 but a later stage still, showing large numbers of virus rods free in the ring zone. × 15,000.

Fig. 9. More advanced stage showing precipitated and contracted polyhedral protein deposited round the virus rods in the ring zone. × 12,100.

### PLATE XI

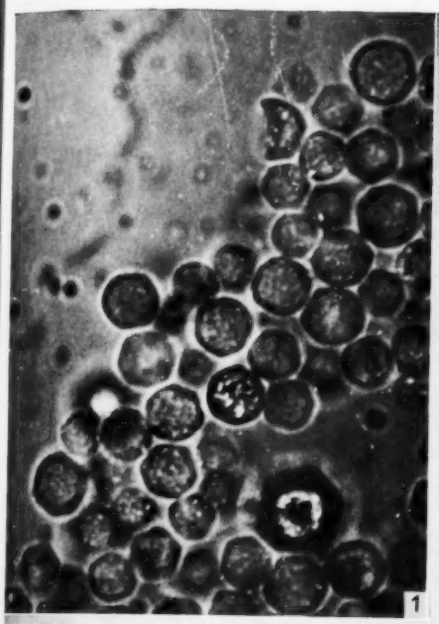
Fig. 10. Section showing several polyhedra formed in the nucleus from polyhedral protein; at the extreme right and lower left hand, virus rods can be seen in the process of being embedded. × 22,500.

Fig. 11. Section of nucleus showing completed polyhedra, the nuclear sap is again free of polyhedral protein and virus rods. × 12,900.

### PLATE XII

Fig. 12. Highly magnified portion of section through central chromatic body of nucleus, showing osmic acid-type fixation, at early stage of polyhedral formation. Note the fine fibrillar structure with several bodies similar to normal virus rods. × 24,600.

Fig. 13. Section of osmic type fixed nucleus, before formation of polyhedra, numerous virus rods visible. × 12,500.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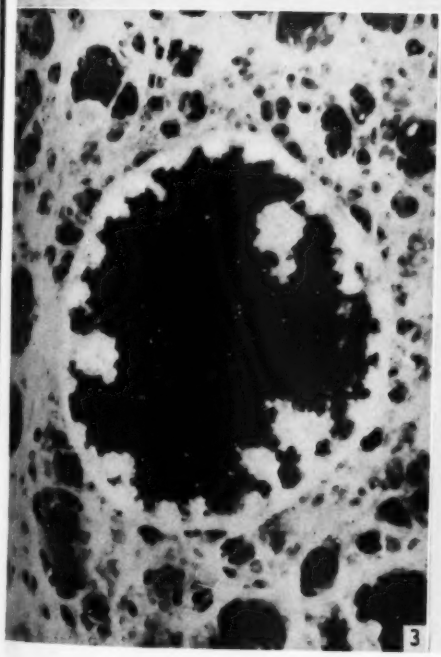
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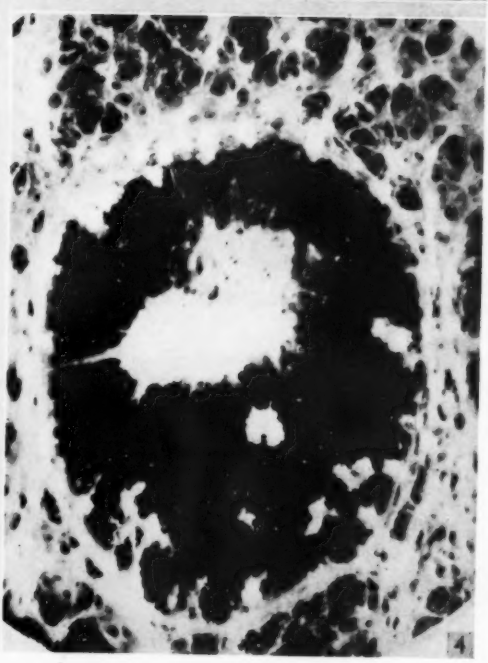
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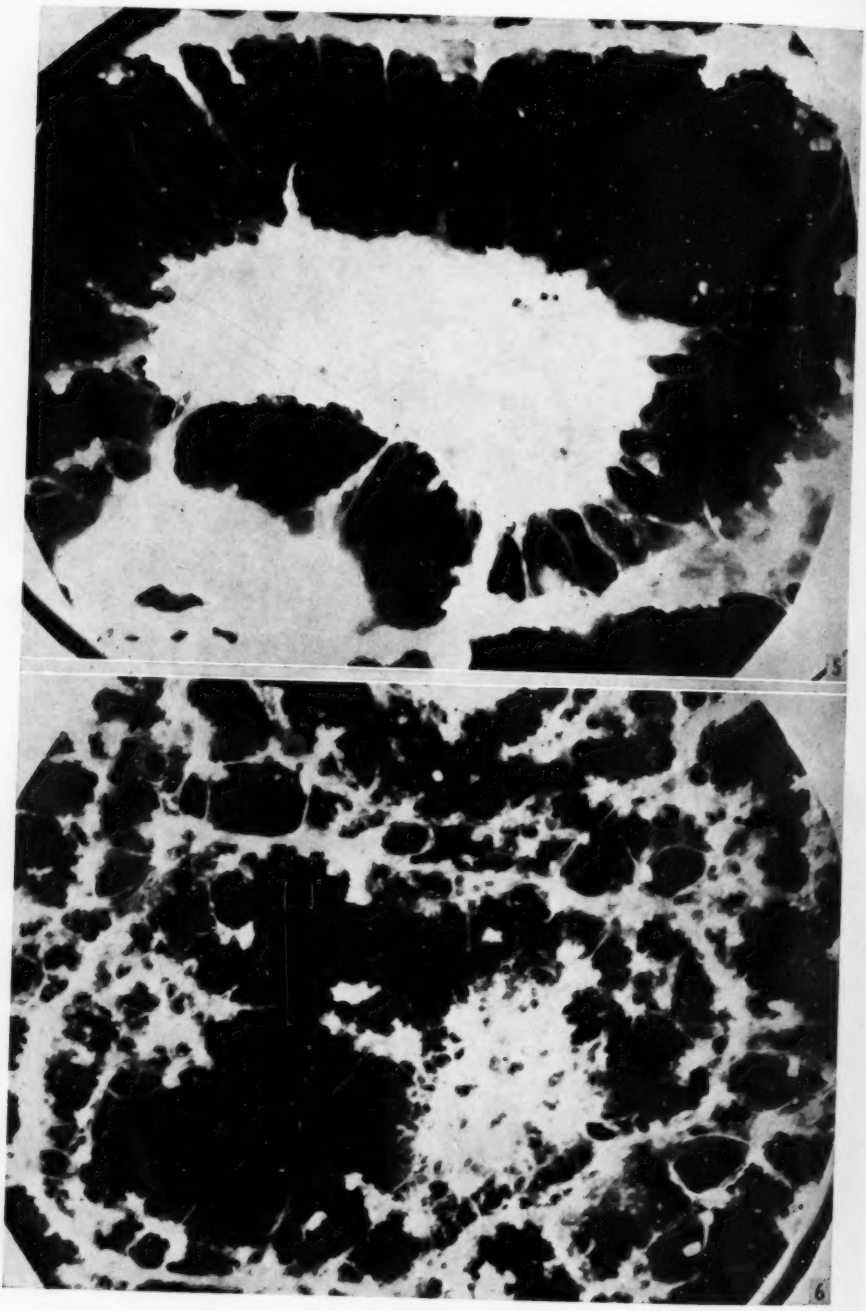

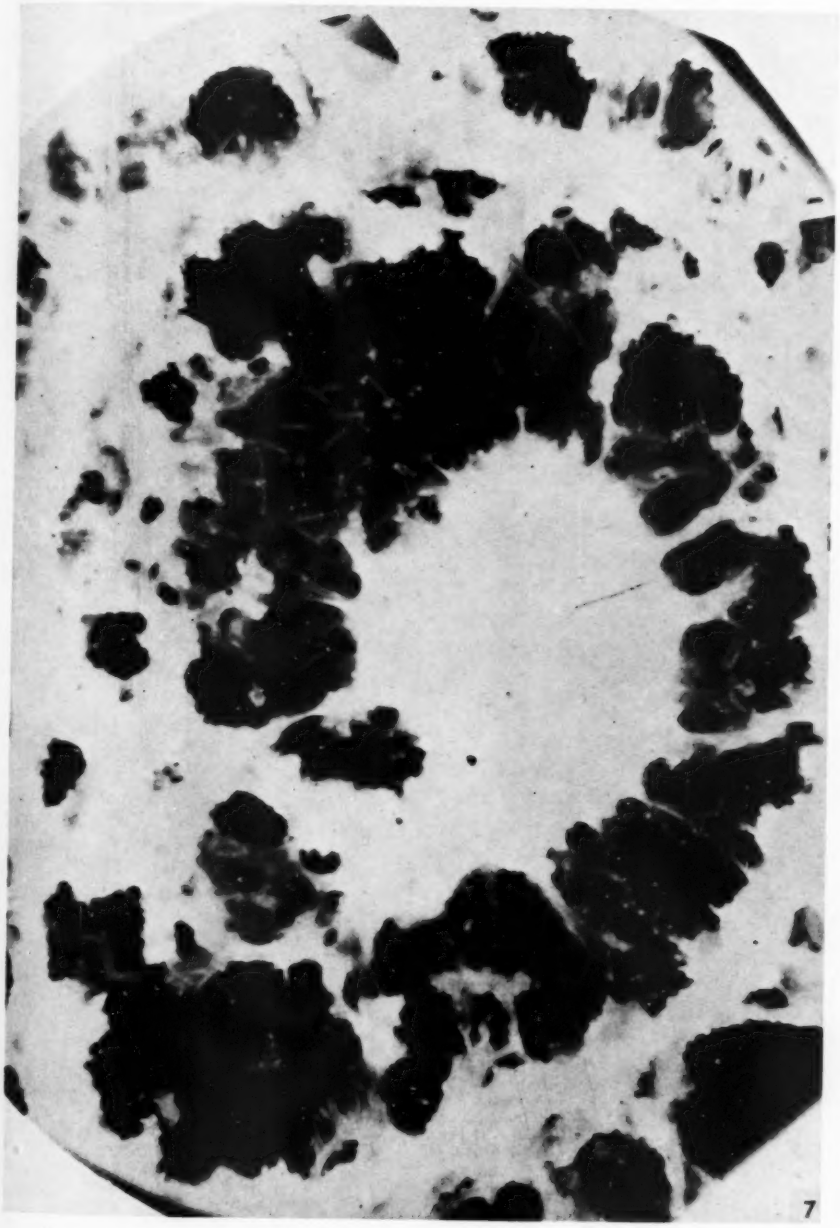
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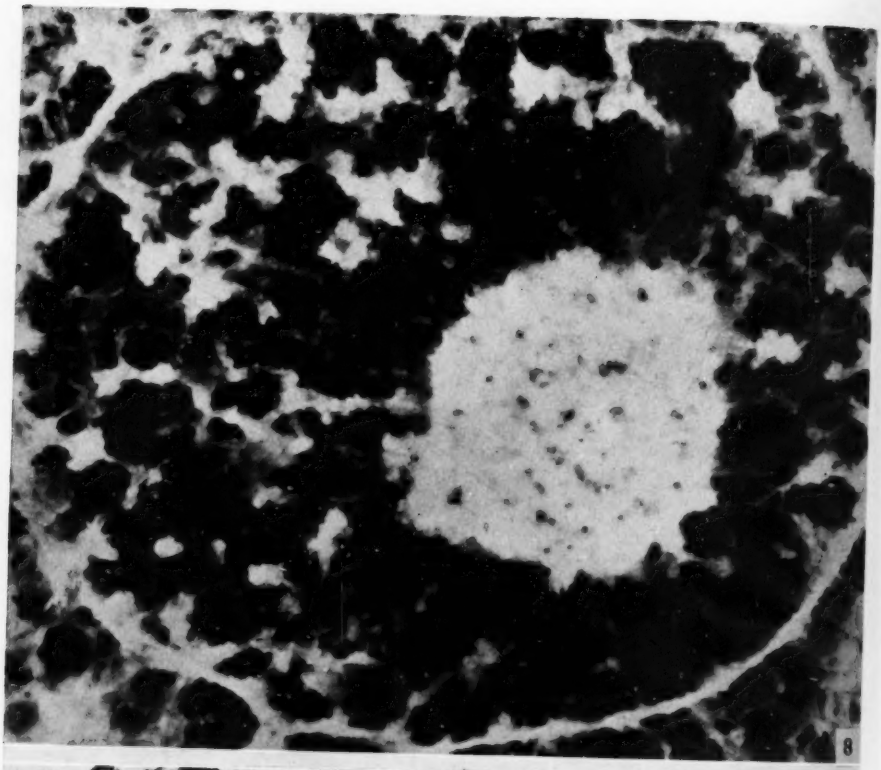
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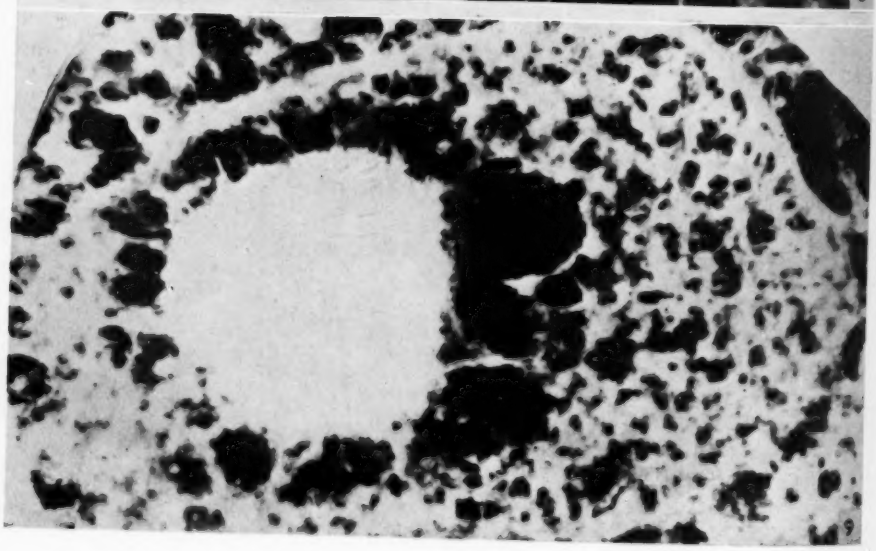
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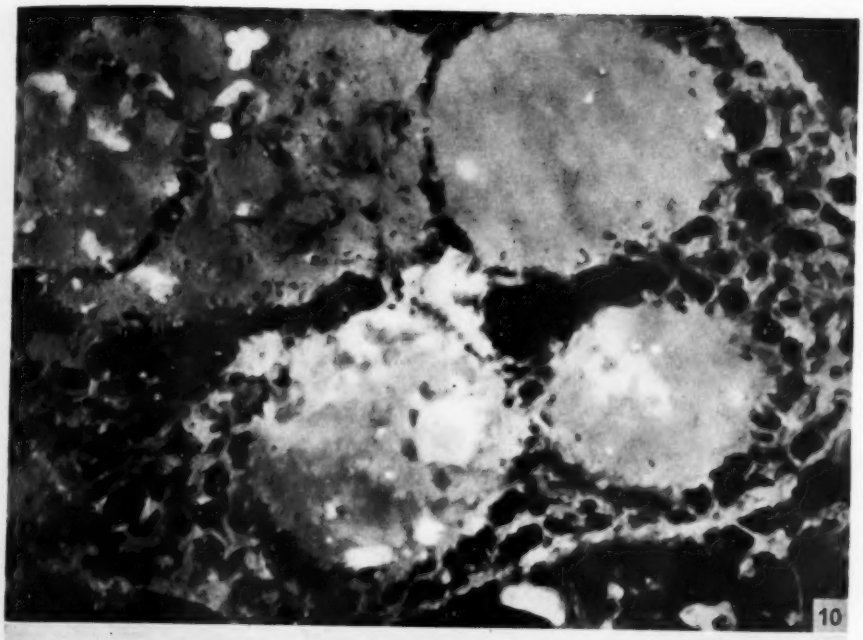
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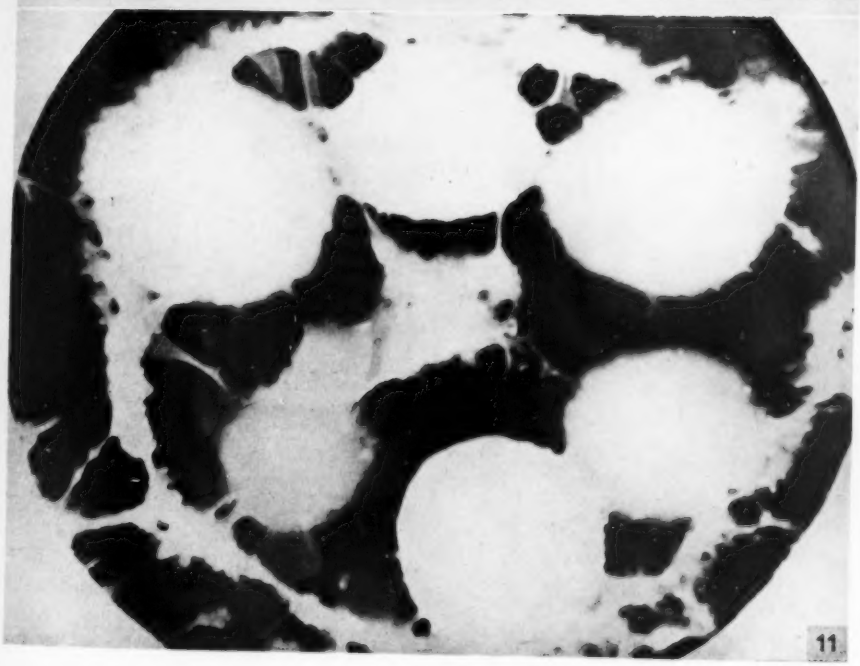
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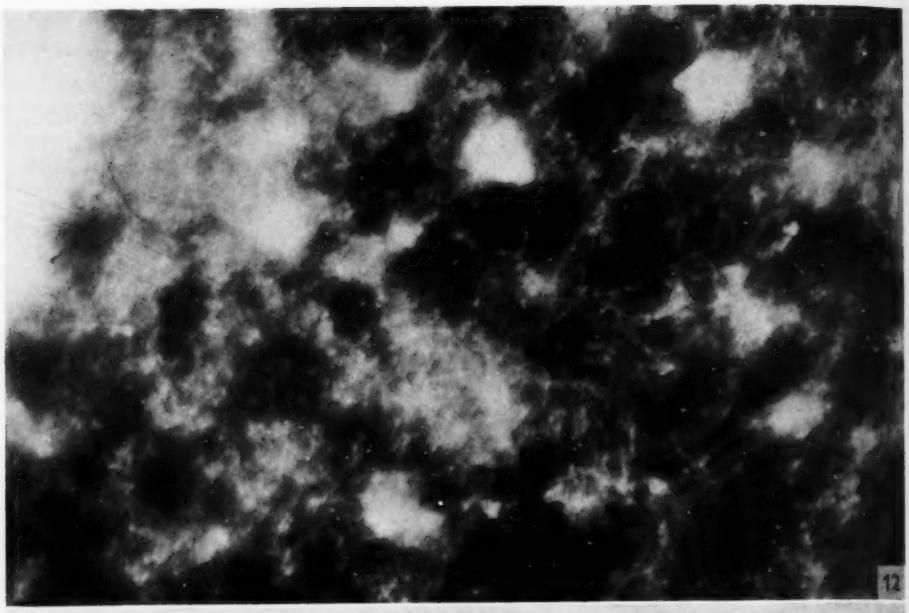
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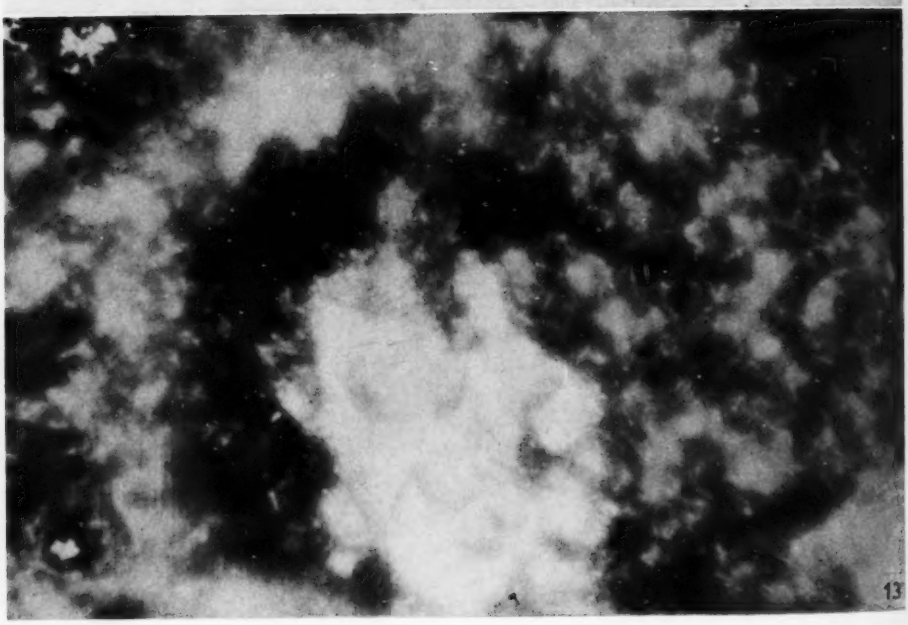
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# ANAEMIA IN MICE CAUSED BY *EPERYTHROZOON COCCOIDES* (SCHILLING, 1928)

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(With 11 Figures in the Text)

*Eperythrozoon coccoides* is a wide-spread parasite of laboratory mice. The infection is usually latent but may be evoked by splenectomy. The parasites then become very numerous in the blood for several days, after which the number decreases and a low, chronic infection ensues. *E. coccoides* was reported as causing only slight anaemic changes in mice, whereas eperythrozoa that are found in sheep, cattle and swine cause severe anaemia in these animals. The anaemia caused by *E. suis* in swine does not become apparent until the numbers of eperythrozoa have decreased considerably. Therefore the observations that were carried out on mice when there were numerous *E. coccoides* in the blood may have been concluded before anaemic changes occurred.

Preliminary observations showed that considerable anaemia developed in mice after heavy infections with two strains of *E. coccoides*. This paper describes experiments that were carried out to relate the degree of anaemia to the number of eperythrozoa in the blood, to find how long such anaemic conditions persisted, and to dissociate the anaemia caused by the eperythrozoa from effects of splenectomy.

### *Review of literature on anaemic changes caused by eperythrozoa*

Neitz (1937) found that *E. ovis* caused severe anaemia in sheep prior to splenectomy. Within 10 days of infection the red cell count dropped to 1 million and the leucocyte count rose to 20,000, with an increase in the number and proportion of monocytes. Anisocytosis, polychromatophilia, Howell-Jolly bodies and normoblasts appeared but haemoglobin regeneration was slow. Erythrophagocytosis was marked. Haemoglobinuria was noted only once and no haemoglobin was detected in the serum. Bilirubinaemia of the indirect van den Berghe type was present. The anaemia was considered to be due to red cell destruction of undetermined mechanism. Relapses occurred up to 109 days after the primary infection and up to 15 months after splenectomy.

*E. wenyoni* caused similar changes in splenectomized cattle, but only mild anaemia in normal cattle (Neitz, 1940).

According to Splitter (1950), heavy infections of *E. suis* caused epidemics of ictero-anaemia in swine, although mild infections produced no clinical symptoms. In splenectomized pigs, blood values began to fall on the 2nd or 3rd day when the parasites were very numerous, and spontaneous reduction in the number of parasites took place as the anaemia developed. Red cell counts fell to 1–2 million/mm.$^3$,

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haemoglobin to 2–4 g./100 ml. of blood, and packed erythrocyte volume to 4 or 7%. Spontaneous agglutination of the erythrocytes was noted during the acute anaemia. White cell counts were often unchanged although sometimes increased. Icterus was marked, with an index of 18–25, and faeces were stained with bile.

Splitter (1950) found that *E. parvum* was relatively non-pathogenic to swine: slight anaemia was found in only two out of five infected, splenectomized pigs.

Bruynoghe & Vassiliadis (1929) attributed no anaemic changes to *E. dispar* in dwarf mice (*Mus minutus*) or voles (*Arvicola* = *Microtus, arvalis*).

Tyzzer (1942) found that *E. varians* was pathogenic for grey-backed deer mice (*Peromyscus maniculatus gracilis*), and caused a reticulocytosis which indicated a notable degree of anaemia.

When Weinman (1944) reviewed the literature on *Eperythrozoon coccoides* he commented that 'increased polychromatophilia is the only constant haematological finding, and in this increase the influence of splenectomy alone has yet to be evaluated'. Schilling (1928) and Bruynoghe & Vassiliadis (1929) observed no blood changes. Dinger (1929) found the red cell count of infected mice to be normal but noted an initial eosinophilia. Eliot & Ford (1930) found an increase in polychromatophilia, and commented that this was evidence for slightly increased blood destruction. McCluskie & Niven (1934) showed that the red cell count fell from 6·5 to 5·0 million/mm.$^3$ when the eperythrozoa were most numerous; the haemoglobin fell to 80% and the number of reticulocytes increased. These workers did not observe any leucocytosis, whereas Eliot & Ford (1930) described a transient slight increase in the number of neutrophils and monocytes when parasites first appeared in the blood.

The course of infection of the related organism *Bartonella* (= *Haemobartonella*) *muris* in mice as described by Adler (1930*a*, *b*) is also relevant to this survey. The number of parasites increased for 3–4 days and then suddenly decreased, thus behaving similarly to eperythrozoa. The red cell count fell from 9·0 to 2·0 million or less per mm.$^3$, and then signs of red cell regeneration became evident if the mouse recovered. The commencement of a remission was accompanied by a mononucleosis of up to 18%, with or without leucocytosis. Haemoglobinuria was detected only rarely in mice, although it was frequently found in infections in rats. The blood of rats taken at the height of infection showed increased coagulability, and haemoglobinaemia was common. No haemolysins were detected. Splenectomy of normal mice caused a temporary decrease in the number of red cells, and an increase in the number of platelets, often four-fold on the second day after splenectomy.

## METHODS AND RESULTS

The 'Mill Hill strain' of *Eperythrozoon coccoides* was isolated from the 'Parkes' albino mice at the National Institute for Medical Research, Mill Hill, and the 'Molteno strain' of *E. coccoides* was isolated from the black and brown mice at the Molteno Institute, Cambridge. The V.S. strain of mice from Mill Hill was used in many experiments as these mice were found to be free of eperythrozoa.

Mice were inoculated with infected blood to ensure heavy infections, as natural infections were very variable in intensity.

Latent infections of *Haemobartonella muris* were occasionally evoked by splenectomy of rats and mice. The coccoid bodies were slightly smaller than eperythrozoa and were usually arranged in straight or branched rows across the erythrocytes, quite unlike the distribution of eperythrozoa. The investigations described in this paper were on animals with pure infections of *Eperythrozoon coccoides*, and care was taken to avoid mixed infections.

Blood of several series of mice was examined at frequent intervals for a period of up to 230 days. Counts of red blood cells, white cells, reticulocytes and haemoglobin were made on each occasion from blood taken directly from the cut tails of the mice. Eperythrozoa were recorded as the number associated with 100 red blood cells, counted from thin films stained with Giemsa's solution. The counts are shown graphically in Figs. 1–10, except that the haemoglobin estimations have been omitted. In order to simplify the graphs, the points marking some of the counts taken during the first 3 weeks have been omitted when they fell upon the line of the curve. During the later stages of infection, when there was little change in the blood values, considerable portions of the graph have been omitted. These omissions are marked by the vertical broken lines. Blood examinations were made at infrequent intervals during the later stages of infection, to prevent undue damage to the tails of the mice. Many relapses were probably missed by infrequent examination because, at this stage, eperythrozoa were numerous in the blood for only 1 or 2 days at a time. The continuance of anaemic conditions was probably related to more numerous relapses than is indicated on the graphs.

### *The anaemia caused by* Eperythrozoon coccoides, *and by splenectomy alone*

*Experiment* 1

Four adult V.S. mice were splenectomized. Mouse 1 was uninfected and was kept separate from the others. Mice 2–4 had been inoculated with Mill Hill *E. coccoides* 1 month earlier, and so their red cell counts were slightly lower than mouse 1 at the beginning of the experiment.

*Mouse* 1 (Fig. 1). Splenectomy alone caused a decrease in the number of red cells from 9·7 to 5·8 million/mm.$^3$, with a corresponding decrease of haemoglobin from 13·0 to 8·4 g/100 ml. A considerable recticulocytosis accompanied the subsequent increase of red cells. White cell counts were also higher than normal. Recovery was continuous from 33 days after splenectomy until 90 days, when the blood values were almost normal. The mouse was then inoculated with *E. coccoides*. Within 30 days the red cell count fell to 3·8 million and the haemoglobin content to 7·0 g. Eight days later the mouse died. The reticulocytes initially increased in number to replace the destroyed red cells, but on the day before death occurred they had decreased in number, and presumably death resulted from this failure of the blood-forming tissues. There was slight leucocytosis during the phase of acute anaemia.

*Mouse* 2 (Fig. 2). Eperythrozoa were very numerous 3 days after splenectomy, and by the 7th day the red cell count had fallen to 3·9 million. The number of

reticulocytes then increased until over 30% of the red cells were immature. The total red cell count began to rise, but with continued parasitaemia it fell again, reaching 1·5 million 33 days after splenectomy. The haemoglobin content was then 4·0 g./100 ml. The number of eperythrozoa decreased and the red cell count rose slowly to 5·0 million, but during a relapse at 130–145 days it decreased to 3·5 million. There was a good reticulocyte response, 48%, and the red cell count rose to

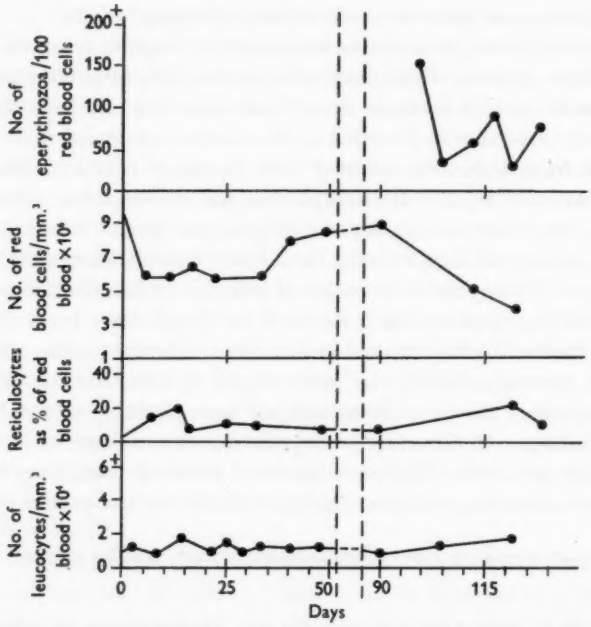


Fig. 1. Mouse 1. Splenectomized on day 0. Infected on day 90. Died on day 130.

Figs. 1–10. Relation of infection with *E. coccoides* to red cell counts, reticulocyte counts and leucocyte counts in mice 1–9 and mouse 14. Vertical broken lines indicate that a portion of the horizontal time axis has been omitted.

7·0 million as the eperythrozoa again became fewer. Two intervening pregnancies possibly affected the blood values. The number of eperythrozoa did not increase unduly during the pregnancies: neither did it decrease, showing that the spleens of the embryos did not affect the course of infection.

The number of white blood cells increased rapidly at the beginning of the first remission and a high, though fluctuating, white cell count was maintained throughout most of the investigation.

*Mouse* 3 (Fig. 3). The initial counts of eperythrozoa after splenectomy were lower than in mice 2 and 4, and the decrease in number of red cells during the first 5 days was not as great. The proportion of reticulocytes increased to 28% on the 6th day, and it seemed as though blood values would rapidly return to normal. However, eperythrozoa persisted in the blood in moderate numbers and the red cell count fell again, reaching 2·0 million by 33 days, with haemoglobin content

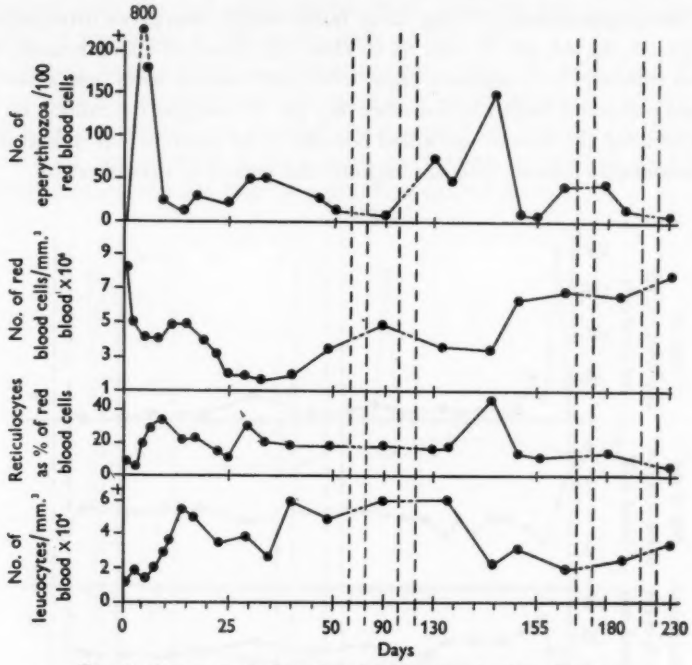


Fig. 2. Mouse 2. Infected and splenectomized on day 0.

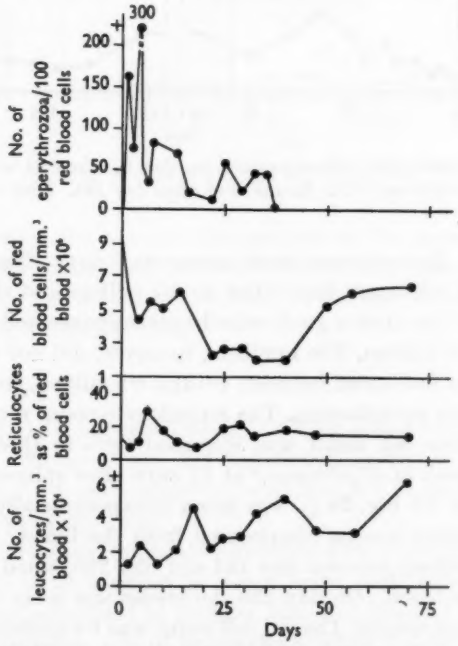


Fig. 3. Mouse 3. Infected and splenectomized on day 0. Treated with neoarsphenamine from day 35.

of 4·8 g. Neoarsphenamine, 2·0 mg./20 g. body weight, was given intraperitoneally on days 35, 37, 43, 44, 45, 61 and 63 to clear the blood of eperythrozoa, and the mouse was removed to a separate cage. No eperythrozoa were seen after day 35, and the red cell count began to rise after day 40. It reached 6·3 million on day 71, but 2 weeks later the mouse was killed because of an intercurrent infection. There was a considerable leucocytosis throughout the period of investigation.

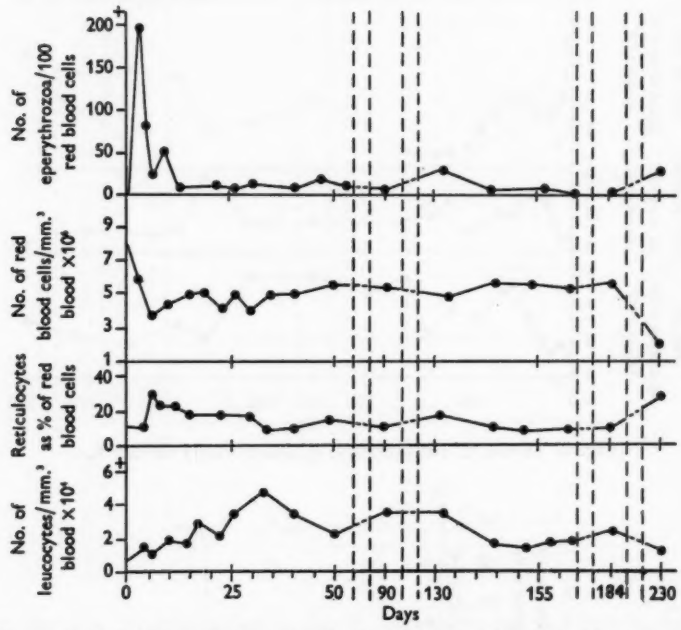


Fig. 4. Mouse 4. Infected and splenectomized on day 0. Treated with neoarsphenamine between days 157 and 175. Re-infected after day 184. Died on day 230.

*Mouse* 4 (Fig. 4). Eperythrozoa were numerous 2 days after splenectomy. By the 5th day the red cell count had fallen to 3·7 million and the haemoglobin to 5·9 g./100 ml. There was then a good reticulocyte response and the number of red cells increased to 5·5 million. The numbers, however, did not continue to rise to the normal value but fluctuated between 4·0 and 5·5 million, corresponding with a fluctuating, low-grade parasitaemia. The reticulocyte count remained higher than normal, and the white cell count was increased two- to three-fold, following a decrease from the peak of 47,000/mm.³ at 33 days after splenectomy.

Neoarsphenamine, 2·0 mg./20 g., was given intraperitoneally on days 157, 161, 165 and 174, and eperythrozoa disappeared from the blood. Unfortunately the mouse was not examined between day 184 and day 230, when eperythrozoa were again present in the blood. On day 230 the mouse was in an extremely anaemic condition and had lost weight. The red cell count was 1·8 million, haemoglobin was 2·3 g./100 ml., and the reticulocyte count was 30%. It was difficult to stop the

flow of blood from the tail after examination, and this loss of blood probably contributed to the death of the mouse later on day 230.

Mouse 4 may have suffered a relapse between days 184 and 230. It appears more likely that the neoarsphenamine cured it of eperythrozoa for a few weeks and it was then very susceptible to re-infection (compare with mouse 1). As it was not separated from the infected mice it may have been re-infected by blood contamination between the cut tails at a previous examination.

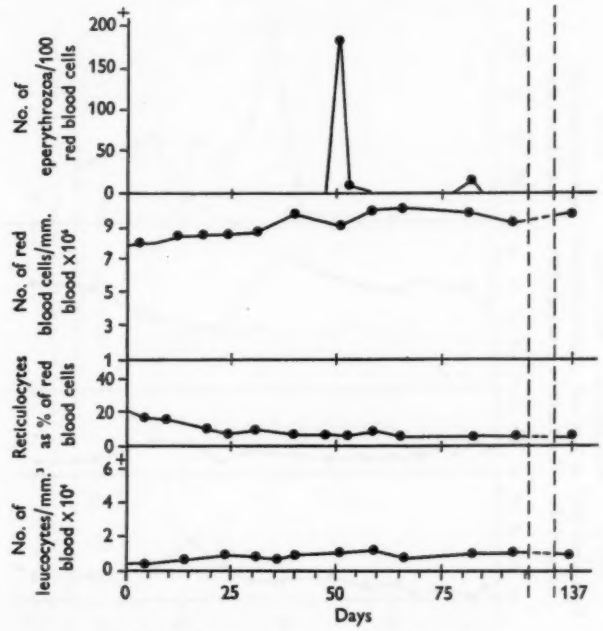


Fig. 5. Mouse 5. Infected on day 46.

As neoarsphenamine did not prevent anaemia in this mouse, it is evident that the increased red cell count in mouse 3 that followed treatment with neoarsphenamine was caused by the action of the drug on the eperythrozoa, and not by its action on the blood-forming tissues.

*Experiment* 2

This experiment was commenced when five litter-mates of uninfected V.S. stock were 3 weeks old. Mouse 5 was left uninfected, mouse 6 was uninfected but splenectomized, mice 7–9 were inoculated with Mill Hill *E. coccoides*, mouse 9 being splenectomized at the same time.

*Mouse* 5 (Fig. 5). It was thought that frequent withdrawal of blood for examination might in itself cause anaemia in young mice, and therefore this uninfected mouse was bled and examined in parallel with the infected mice. There was no evidence of such anaemia, nor of leucocytosis which might have resulted from

inflammation at the cut tip of the tail. Because the mouse was very young at the beginning of the experiment, the counts did, in fact, show the rate at which the number of red blood cells increased and the percentage of reticulocytes decreased until adult values were reached when the mouse was about 9 weeks old. The white cell count increased slightly during this period.

Mouse 5 was inoculated with *E. coccoides* on day 46. There was a transient acute parasitaemia accompanied by a decrease in red cell count and followed by a slight

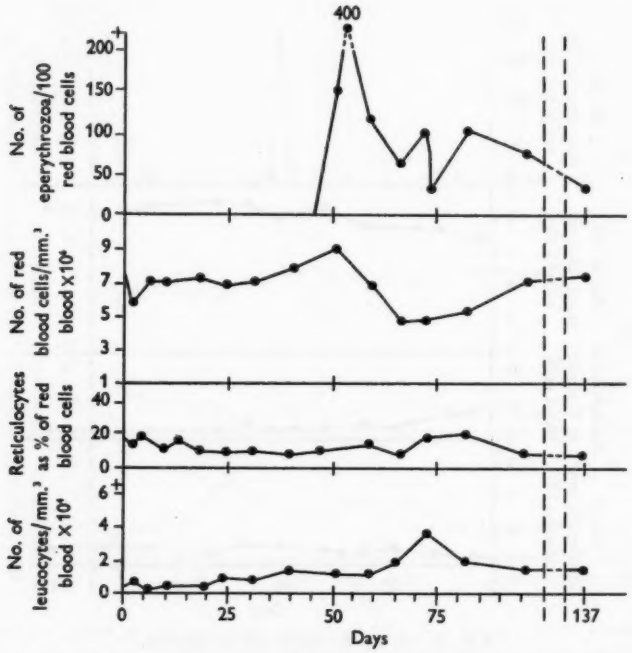


Fig. 6. Mouse 6. Splenectomized on day 0. Infected on day 46.

rise in reticulocytes and leucocytes. Blood values returned to normal, but eperythrozoa were occasionally seen and the red cell count fell again from 10 to 9 million.

*Mouse* 6 (Fig. 6). Splenectomy alone caused a decrease in the red cell count from 7·3 to 5·7 million on the 2nd day. Recovery was rapid and continued progressively until the adult count was reached. This was, however, 10 days after it had been reached in mouse 5, and both the reticulocyte and leucocyte counts were higher than in mouse 5.

Mouse 6 was infected on day 46. Eperythrozoa became numerous in the blood and a moderate number persisted during the investigations. The red cell count dropped to 4·5 million, haemoglobin 8·4 g., on day 66, but then rose slowly to 7 million despite the eperythrozoa. The beginning of the recovery was marked by an increase in reticulocytes and leucocytes.

The anaemic changes observed in mouse 6 were not as great as those found in mouse 1. This is probably because mouse 6 was very young and its erythropoietic

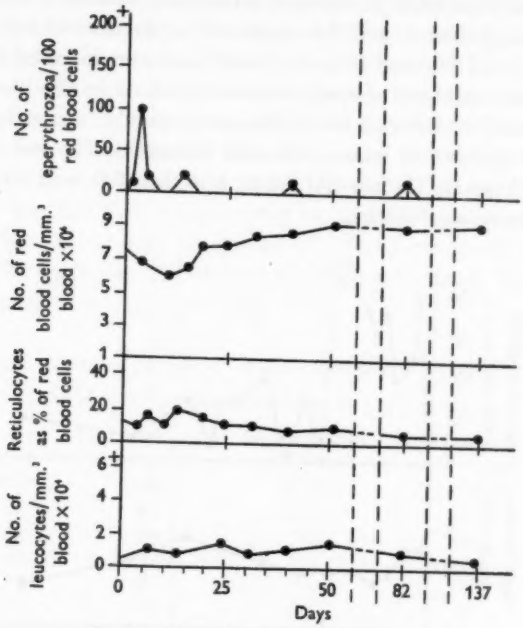


Fig. 7. Mouse 7. Infected on day 0.

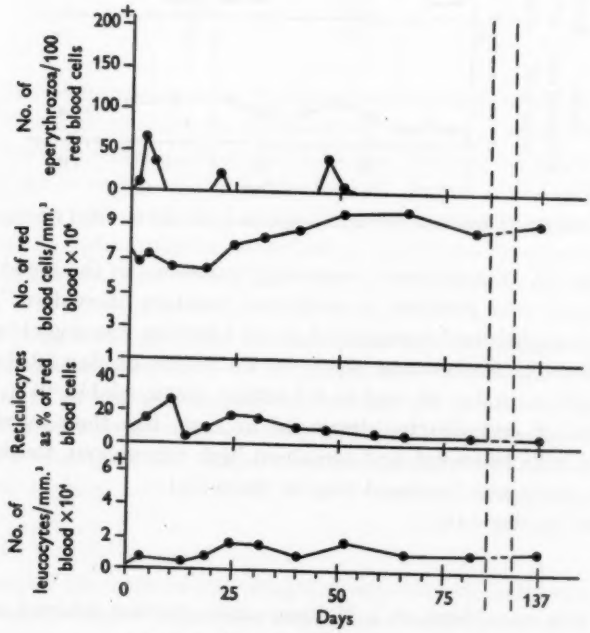


Fig. 8. Mouse 8. Infected on day 0.

system more labile than that of mouse 1, which was at least 2 months old at the beginning of the experiment, and 5 months old at the time of infection.

*Mouse* 7 (Fig. 7) *and Mouse* 8 (Fig. 8). These mice were infected but not splenectomized. Moderate numbers of eperythrozoa appeared in the blood and the red cell counts decreased to 6·0 and 6·4 million respectively, while the percentage of reticulocytes and number of leucocytes were increased. The red cell counts rose until they reached nearly the normal value, but then fell, and remained between 8 and 9 million for several weeks.

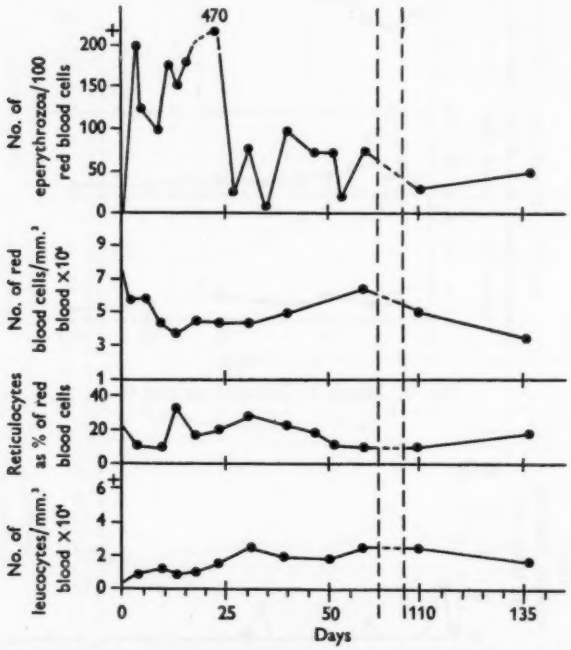


Fig. 9. Mouse 9. Infected and splenectomized on day 0. Died on day 148.

*Mouse* 9 (Fig. 9). Eperythrozoa were very numerous in the blood for 24 days after splenectomy and persisted in moderate numbers thereafter. The red cell count decreased rapidly and remained at about 4 million, haemoglobin 6·0 g., from day 13 until day 31. It then rose slowly to 6·7 million on day 59, but decreased again to 5·1 million on day 96, and to 3·5 million, haemoglobin 49 g., on day 137. The proportion of reticulocytes decreased at first, but then increased as the number of red cells increased and remained high throughout the investigation. The leucocyte count was increased two- to three-fold.

Mouse 9 died on day 148.

*Experiment* 3

A litter of five mice, born to a Molteno strain mother infected with Molteno *E. coccoides*, was examined from 5 days after birth.

*Mouse* 10 had a very heavy infection on day 5, 811 eperythrozoa/100 red cells, which decreased to 100 on days 6 and 7, and fifteen on day 9. Very few eperythrozoa were found subsequently. *Mouse* 11 was splenectomized 9 days after birth, and inoculated with Molteno *E. coccoides* on day 13. Eperythozoa were 500/100 red cells on days 19, 20 and 21, and between 100 and 200 on days 23-28. *Mouse* 12 was inoculated on day 16 with Mill Hill *E. coccoides* and a light infection ensued, with 5 eperythrozoa/100 red cells on days 19 and 20, and 15 on day 21. *Mouse* 13 was inoculated with the Mill Hill strain on day 19, and 350 eperythrozoa/100 red cells were found on day 21, but only ten on day 22. No eperythrozoa were found in the blood of *Mouse* 14 up to day 27, when it was splenectomized. Parasites were very numerous in the blood 6 days later (Fig. 10).

Table 1. *Blood values of five litter-mates examined* 27 *and* 40 *days after birth*

(See text for details of infection and splenectomy.)

| | Mouse | Red blood cells $\times 10^6$/mm.$^3$ blood | Haemoglobin (g./100 ml. blood) | Reticulocytes as % of red blood cells | Leucocytes $\times 10^3$/mm.$^3$ blood |
|---|---|---|---|---|---|
| Day 27 | 10 | 5·1 | 9·1 | 20 | 7·0 |
| | 11 | 3·6 | 7·0 | 22 | 13·0 |
| | 12 | 5·4 | 8·8 | 9 | 7·3 |
| | 13 | 4·1 | 7·4 | 14 | 11·2 |
| | 14 | 5·3 | 9·1 | 9 | 10·3 |
| Day 40 | 10 | 7·5 | 12·2 | 12 | 15·0 |
| | 11 | 2·8 | 4·2 | 28 | 55·2 |
| | 12 | 6·0 | 5·1 | 18 | 8·7 |
| | 13 | 6·0 | 5·1 | 14 | 7·0 |
| | 14 | 4·1 | 7·7 | 14 | 17·5 |

Blood counts performed on mice 10–14 on days 27 and 40 are recorded in Table 1. *Mouse* 10 had a high reticulocyte count on day 27, but otherwise showed no sign of its early heavy infection. *Mouse* 11 had a very low red cell count and increased reticulocyte and leucocyte counts on both days. *Mouse* 12 showed slight signs of its light infection. *Mouse* 13 had a low red cell count and increased reticulocyte count. *Mouse* 14 had normal blood values on day 27, but the number of red cells decreased and the reticulocyte and leucocyte counts increased by day 40, following splenectomy and an acute infection of *E. coccoides*.

*Mouse* 14 (Fig. 10). The blood of this mouse was subsequently examined for 182 days after splenectomy. The red cell count fluctuated between 4 and 6 million throughout most of this period, and the reticulocyte count was at a moderately high level. The white cell count was very high from day 40 onwards. Few eperythrozoa were found in the later stages of infection, and the blood values on day 182 had improved slightly, but remained at the same level on day 202.

*Experiment* 4

As only two of the mice in the preceding experiments had been infected with Molteno *E. coccoides*, two further mice were infected with this strain of eperythrozoa.

*Mouse* 15 had a heavy infection (600 eperythrozoa/100 red cells) on the 4th day

after splenectomy and the red cell count had then fallen from 9 million to 7·3 million/mm.³. On the 7th day the red cell count was only 5·0 million, haemoglobin 8·0 g./100 ml., and the white cell count was 38,000; eperythrozoa had decreased to 100/100 red cells.

*Mouse* 16 developed only a light infection after splenectomy; eperythrozoa varied between 50 and 150 per 100 red blood cells during the first week, and the

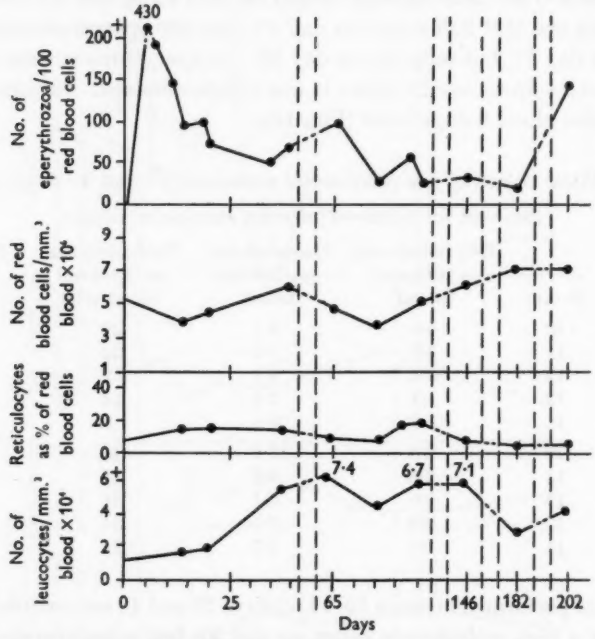


Fig. 10. Mouse 14. Course of infection after splenectomy on 27th day after birth. Day of splenectomy is shown as day 0 in graph.

red cell count dropped to only 8·3 million/mm.³. A moderate infection of eperythrozoa persisted in the blood, and on the 26th day after splenectomy the red cell count was 7·5 million, reticulocytes 9 %, and white cells 42,000/mm.³. On day 42, after an increase of eperythrozoa to 100/100 red cells, the red cell count had fallen to 6·0 million, reticulocytes were 11 %, and white cells 20,500.

### *Studies on the chemical and physical properties of blood, urine and faeces of mice infected with* Eperythrozoon coccoides

These tests were on specimens from mice with heavy primary infections of *E. coccoides* and from mice with long-established chronic infections. As only small quantities of material could be obtained, the tests were carried out on a very small scale and were frequently somewhat modified. The techniques were taken from the section by Clough in Stitt, Clough and Branham (1948). Serum was obtained from oxalated blood and from blood that had been left to clot at 37° C. for 4 hr. Urine and faeces were collected in test-tubes directly from individual mice.

*Coagulation of blood*

Blood containing numerous eperythrozoa frequently coagulated when taken into oxalated tubes or into an equal volume of citrate saline, or while being diluted with Hayem's fluid for red cell counts. This has previously been described by Adler (1930*a*) as an effect of *Bartonella* (= *Haemobartonella*) *muris*, and by Splitter (1950) in *Eperythrozoon suis* infections. No haemagglutinins were detected, for serum taken from heavily infected blood did not agglutinate washed red cells.

Coagulation also occurred more rapidly than normal. Blood was taken from the cut tails of mice into capillary tubes and was blown out at minute intervals. Coagulation was said to begin when a thin clot was first ejected, and to be complete when it was almost impossible to dislodge the completely clotted blood from the capillary tubes. Normal blood began to clot at 8 min. and was completely clotted at 10–11 min. (four mice). Blood containing numerous eperythrozoa, tested 7 days after splenectomy, began to clot after 4 min. and was completely clotted at 5–6 min. (three mice). Blood from one uninfected mouse that had similarly been splenectomized 7 days previously began to clot at 7 min. and was completely clotted at 9 min. This was slightly more rapid than normal blood, but was much less rapid than infected blood. Therefore the effect found with *E. coccoides* was caused by the parasite and was not merely an effect of splenectomy. The clotting-time of blood containing fewer than 4 million red cells/mm.$^3$ was considerably increased, despite the presence of moderate numbers of eperythrozoa.

*Fragility of red blood cells*

Blood was taken directly from the cut tails of mice into red cell diluting pipettes, and was diluted with hypotonic solutions of sodium chloride that ranged from 0·7 to 0·3 %. The pipettes were left at room temperature for 1 hr. Red cell counts were then made on a haemacytometer slide and were compared with counts made on blood diluted with Hayem's fluid and examined at once. The degree of haemolysis in the different salt solutions is shown in Fig. 11. Lysis of red cells from normal mice occurred between 0·4 and 0·6 % saline (average of three mice). Blood cells from newly weaned mice (two mice) were more fragile and red cells taken from infected, splenectomized mice during the initial decrease in number of erythrocytes were still more fragile, as lysis occurred between 0·5 and 0·7 % saline (average of four mice).

It was thought that reticulocytes might be more fragile than mature erythrocytes and that the increased fragility during infection was caused by the reticulocytosis. However, the newly weaned mice had reticulocyte counts of 20 % and only one of the infected mice had as high a count, yet the blood cells from the infected mice were more fragile than those from the baby mice. Therefore, reticulocytosis is not the only cause of the fragility in hypotonic saline.

No haemolysins were detected in serum from infected mice when it was added to washed red cells. Adler (1930*a*) similarly did not detect any with *Haemobartonella muris* in rats. The effect on the red blood cells of the infected host appears to be caused directly by the eperythrozoa that are massed upon them. When large

numbers of eperythrozoa were found on one cell (60–70 have often been counted), that cell frequently appeared distorted and paler or ghost-like in stained blood films, and also less dense when viewed by phase-contrast microscopy. These findings suggest that the eperythrozoa absorb the contents of the red blood cells when they settle upon them.

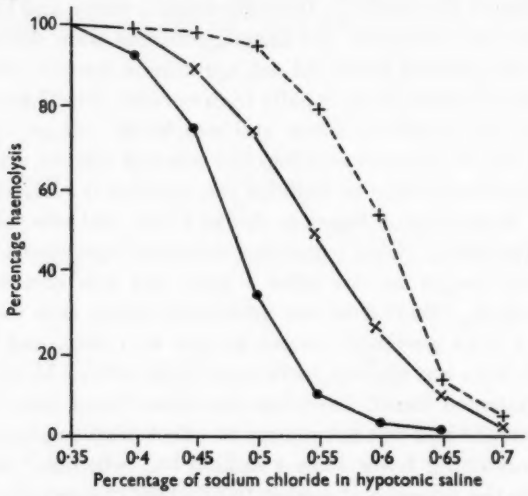


Fig. 11. The percentage haemolysis of red blood cells from normal and infected mice in hypotonic saline. Normal mice, ●——●; newly weaned mice, ×——×; infected, splenectomized mice, + – – – +.

*Van den Berghe test on serum*

Serum taken during the period of haemolysis gave no colour change in the direct or indirect van den Berghe test (Clough, p. 871), even with the addition of caffeine-sodium salicylate. That is, no bilirubin was detected.

*Tests on urine*

The colour of the urine varied considerably in normal mice.

The benzidene test (Clough, p. 836) on an acidified ether extract of urine from heavily infected mice was always negative—that is, no haemoglobin was present.

When urine was layered over alcoholic iodine solution no green colour appeared, i.e. no bile pigments were detected (Clough, p. 835). The Huppert-Nakayama test for bile pigments (Clough, p. 835) was also negative, as was Schlessinger's test for urobilin (Clough, p. 836). When urine was tested with *p*-dimethylaminobenzaldehyde (Clough, p. 835) for urobilinogen, a deep yellow colour was obtained from infected and some normal mice. There was, therefore, little increase of urobilinogen.

A bright red colour was occasionally obtained with Ehrlich's diazo reaction (Clough, p. 836) in both infected and normal mice. It was probably caused by an intercurrent infection and not by the infection with *E. coccoides*.

A little albumin was detected in the urine of normal mice, but the albumin content of the urine from mice with chronic infections of *E. coccoides* was considerably increased. A thick white ring appeared at the interface when urine was

layered over concentrated nitric acid (Heller's test, Clough, p. 827). The ring still appeared if the urine was diluted with an equal volume of water. No ring appeared when the urine was layered over a strong solution of citric acid (75 g. in 100 ml. water). That is, 'pseudo-albumin' was not present (Ramsden, 1928).

Urine was tested in the cold with glacial acetic acid; no precipitate appeared, indicating the absence of mucin (Clough, p. 826). The precipitate that formed on heating the urine persisted and slightly increased when glacial acetic acid was added, showing the presence of albumin. Purdy's modification of this test was also positive (Clough, p. 827).

*Tests on faeces*

A suspension of faeces was shaken with ether to remove fats. The residue was then acidified and a further ether extract was tested with benzidine and hydrogen peroxide (Clough, p. 856). Faeces from normal and infected mice occasionally gave the colour reaction of haemoglobin; the excretion of haemoglobin by this route was, therefore, not increased during the anaemia.

Faeces were ground up with a saturated solution of mercuric chloride and left for 3 hr. Normal faeces were then a dark straw colour while faeces from infected mice had a salmon pink coloration, indicating an increase in urobilin (Clough, p. 855). No green colour indicative of bilirubin was detected.

*The effect of* Eperythrozoon coccoides *on the blood picture in mice*

It has been shown above that during infections of *E. coccoides* a marked reticulocytosis followed each decrease in red cell count and occurred when the eperythrozoa decreased in number. The reticulocytes of mice are larger than the mature red cells, their diameter being 6–7$\mu$ compared with 5–6$\mu$. During the anaemic and regenerative phases of *E. coccoides* infection there was an increase in the number of macrocytes due in part to the increased reticulocyte count and in part to the larger size of some mature red cells, up to 7·5$\mu$ diameter, and of some reticulocytes, up to 8·0$\mu$ diameter. The Price-Jones curves for the infected mice were flatter than normal and spread further along the base-line.

Although the number of reticulocytes was increased, normoblasts were rarely seen, and there was little or no increase in the number of Howell-Jolly bodies.

In differential white cell counts from normal mice the lymphocytes numbered from 10 to 33% and the polymorphonuclear granulocytes from 50 to 65%. Immature white cells accounted for the remainder, myelocytes numbering 6–12% and larger cells from 7 to 16%. Immature white cells containing two nuclei were rarely seen in normal blood films, but in mice infected with *E. coccoides* 1–6% of the white cells were such binucleate cells. The number of myelocytes also increased to 20–30% in infected mice, but the percentage of larger cells remained approximately normal. Very large cells with numerous vacuoles and containing large red-staining granules (probably phagocytized eperythrozoa) were found at the height of acute infections. Degenerate polymorphonuclears were also found then. When myelocytes were most numerous the polymorphonuclears decreased to 35–50%, and the lymphocyte count was also slightly lower than normal.

All of the immature cells and the ring-stage polymorphonuclears were phagocytic. In chronic infections of the malaria parasite *Plasmodium berghei* they contained large amounts of pigment. No pigment was found in the lymphocytes.

Blood films were stained for peroxidase granules by Beacon's modification of Graham's method (from Whitby & Britton, 1935, p. 485). Numerous granules were found in the mature polymorphonuclears and fewer in the ring forms. Granules were occasionally, although not always, found in the myelocytes and larger cells. No granules were found in the lymphocytes. Some of the mature polymorphonuclears contained granules that all stained a golden-brown colour instead of the usual blue-black colour.

Supravital staining by Sabin's technique (see Whitby & Britton, 1935, p. 487) coloured the numerous granules of mature polymorphonuclears a red colour. Fewer granules were found in the myelocytes, and very few larger cells contained any. Mitochondria, staining green, were more numerous in the immature cells and the lymphocytes than in the mature polymorphonuclears. The immature cells were only very slightly motile.

It appears that, during acute attacks of *E. coccoides*, the granulocyte leucocytes phagocytize eperythrozoa and themselves become degenerate. More of the immature myelocytes are then produced to replace them. It is probable that this increased proliferation of leucocytes continues after the number of eperythrozoa in the blood has decreased; that is, after the phagocytic activity of the granulocytes and their subsequent degeneration have come to an end. This would explain why the leucocytosis has been found to commence at the end of the acute stage of infection.

## DISCUSSION

It has been shown that both Mill Hill and Molteno strains of *Eperythrozoon coccoides* can cause anaemia in V.S. and Molteno mice. Splenectomized mice in which the blood values had returned to normal were very sensitive to *E. coccoides*, and severe anaemia resulted from infection although only slight changes in the blood were detected when adult non-splenectomized mice were infected. In chronic infections the anaemia became more severe at times when eperythrozoa were numerous in the blood, and blood values improved when eperythrozoa were cleared from the blood by neoarsphenamine. It is most unlikely that these effects were dependent upon some haemolytic agent other than *E. coccoides*.

Splenectomy alone caused some anaemia in normal mice, but when recovery took place it was continuous until normal blood values were reached. In contrast, a more severe anaemia developed when mice infected with *E. coccoides* were splenectomized, and blood values did not return to the normal level. Heavy infections were necessary to produce very low red cell counts in splenectomized mice within 1 or 2 weeks. Light or medium infections initially caused only moderate anaemia, but the persistence of such infections caused a further reduction in the red cell count and maintained an anaemic condition for many months.

Newly weaned mice had lower red cell counts than adult mice. When they were infected but not splenectomized the red cell count increased at a much slower rate

than in uninfected mice. Young infected mice developed acute infections and severe anaemia after splenectomy but appeared to be able to make good a decrease in red cells more rapidly than did adult mice.

It is hoped to present data on congenital transmission more fully later. Weinman (1935) and Dinger (1929) found that it apparently did not occur. I have found infection sporadically in very young mice. In the present experiments, mouse 10 had a very heavy blood infection when it was first examined 5 days after birth. The eperythrozoa soon disappeared but a high reticulocyte count remained as evidence of the earlier infection. Infection with *E. coccoides* is probably one of the causes of the differences in the reticulocyte count between different strains of mice and between individuals of an infected strain.

While it is apparently an intrinsic feature of *E. coccoides*, as of other species of *Eperythrozoon*, to destroy red blood cells, mice are able to recover very quickly from anaemia and to make good the loss of red cells by increased production of reticulocytes. The immediate cause of anaemia is apparently the direct action of the eperythrozoa upon the red cells, and the presence of numerous eperythrozoa in the serum seems to be the cause of the rapid coagulation noted during the height of infection. No breakdown products of the red cells were detected by chemical tests; this may be because of differences in metabolism between mice and man. Clinical signs of anaemia only became evident if very large numbers of eperythrozoa were present or if the mice were subject to a prolonged infection. The effect of eperythrozoa upon the white cell count has been discussed in the relevant paragraph in the preceding section.

A chronic haemolytic anaemia in mice has, therefore, been shown to be caused by *Eperythrozoon coccoides*, which, although initially abundant in the blood, has usually decreased considerably in number by the time the anaemic condition first becomes evident. In these experiments, heavy infections of *E. coccoides* were evoked by splenectomy, but concurrent infection with other micro-organisms will also evoke latent infections of *E. coccoides* and the anaemia caused by the eperythrozoa is then probably an unrecognized complicating factor.

## SUMMARY

Experiments were designed to dissociate the effect of *Eperythrozoon coccoides* on the blood of mice from the effect of splenectomy. Both agents caused a decrease in number of red blood cells which was followed by an increased production of reticulocytes. The anaemic condition caused by the eperythrozoa persisted for more than 230 days after splenectomy, and parasites were seen in fluctuating numbers at intervals throughout this period.

At the height of infection, red blood cells were more fragile than normal in hypotonic saline and the blood clotted more rapidly than normal. No breakdown products of haemoglobin were detected in the urine or serum, and no haemolysins or haemagglutinins were detected in the serum. The white cell count was usually increased after heavy infections, the chief increase being in the number of myelocytes and other immature cells.

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# THE CHEMOTHERAPY OF *PLASMODIUM BERGHEI*

## II. ANTAGONISM OF THE ACTION OF DRUGS

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### I. INTRODUCTION

The concept of chemotherapeutic agents acting as metabolite analogues and blocking the normal enzyme systems of cells was introduced by Woods (1940) and Fildes (1940) when they discovered that *p*-aminobenzoic acid antagonized the action of sulphonamides *in vitro*. This antagonism was also demonstrated *in vivo* (Selbie, 1940). *p*-Aminobenzoic acid similarly antagonized the antimalarial activity *in vivo* of sulphonamides against *Plasmodium lophurae* (Marshall, Litchfield & White, 1942; Seeler, Graessle & Dusenbery, 1943), *P. gallinaceum* (Maier & Riley, 1942; Bishop & McConnachie, 1948), and *P. knowlesi* (Richardson, Hewitt, Seager, Brooke, Martin & Maddux, 1946), and also against the sporozoites of *P. gallinaceum* developing in *Aëdes aegypti* mosquitoes (Terzian, Stahler & Weathersby, 1949).

Folic acid (pteroylglutamic acid) antagonized the action of sulphonamides against those bacteria that synthesize this compound from *p*-aminobenzoic acid (Lampen & Jones, 1946) and against *P. gallinaceum*, *in vivo* (Greenberg, 1949*a*). Other compounds which inhibited the antibacterial action of sulphonamides, or which could replace *p*-aminobenzoic acid as a growth factor, included the nucleic acid derivatives thymine, adenine, guanine and xanthine, and a number of amino-acids, methionine being most frequently reported. It was postulated that *p*-aminobenzoic acid probably functions in the biosynthesis of these compounds and that the sulphonamides interfere with its utilization in one or more of these syntheses in susceptible cells. Thymine and the purines could sometimes replace folic acid

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as growth factor or antagonist (Woods, 1950). Another compound which also probably functions in these biosyntheses is folinic acid (Leucovorin, Citrovorum Factor). It can replace xanthine, hypoxanthine or guanine in the growth requirements of *Leuconostoc citrovorum* and can itself be replaced by large amounts of folic acid in the presence of purines (Sauberlich, 1949).

The antimalarial drugs pyrimethamine (Daraprim), proguanil (Paludrine) and the 2:4-diaminopteridines resemble the sulphonamides in being folic acid antagonists. It is generally believed that these drugs interfere with the utilization of folic acid rather than its synthesis. However, Thurston (1950*b*) described the antagonism of proguanil by *p*-aminobenzoic acid, using *P. berghei*, and similar antagonism has now been demonstrated by Greenberg (1953), using *P. gallinaceum*.

This paper describes experiments with *P. berghei* in mice in which attempts were made to antagonize the action of pyrimethamine, sulphadiazine, proguanil and its active metabolite (CPT) which is 2:4-diamino-1-*p*-chlorophenyl-1:6-dihydro-6:6-dimethyl-1:3:5-triazine (Carrington, Crowther, Davey, Levi & Rose, 1951), and three of the series of pteridines that was tested by Collier & Waterhouse (1950, 1952) for vibriostatic and antibacterial activity; these are 0/97 (2:4-diamino-3:7-camphanopteridine), 0/103 (2:4-diamino-6:7-di*n*hexyl pteridine), and 0/164 (2:4-diamino-1′-ethylindolo-2′:3′-6:7-pteridine).

## II. METHODS

Mice were inoculated with 1 million erythrocytes parasitized by *P. berghei* and were treated with drug and antagonist on the day of inoculation and the next 3 days. Blood films were examined on the day after treatment ended, and the degree of infection in the mice that had received drug and antagonist was compared with the degree of infection in the mice that had received the drug alone and in the controls. All the drugs were given intraperitoneally, usually at approximately the minimum effective dose; they were all given once daily, except proguanil which was given twice daily.

The antagonists were given by mouth, twice daily, except in experiments where, as described below, *p*-aminobenzoic acid and folic acid were given subcutaneously, twice daily. The first dose of antagonist was always given at least 5 hr. before the first dose of drug on the day of inoculation. The figures shown in parenthesis after each antagonist in the following results represent the dose of the compound in mg. per kg. of body weight per dose. Synthetic pteroylglutamic acid was used in all experiments for folic acid antagonism. When compounds were insoluble in water they were ground in Griffith's tubes and administered as a suspension in water.

## III. ANTAGONISM OF PYRIMETHAMINE

Hitchings (1952) summarized the work that led to the production of pyrimethamine, 5-(*p*-chlorophenyl)-2:4-diamino-6-ethylpyrimidine. It was one of a series of compounds which were all competitive antagonists of folic and folinic acids in the growth of *Lactobacillus casei*. When tested against *Streptococcus faecalis*, pyrimethamine probably interfered with the conversion of folic to folinic acid. However,

folinic acid did not antagonize the antimalarial action of pyrimethamine against *Plasmodium gallinaceum*.

In the present experiments, the action of pyrimethamine (1·0 mg./kg.) against *P. berghei* was not antagonized by folinic acid, thymine, riboflavin, glycine, histidine or cytosine (each tested in doses of 50 mg./kg.), thiamine (100 mg.) or *d*-ribose (200 mg.). The action of pyrimethamine against *P. gallinaceum* was not antagonized by *p*-aminobenzoic acid, 300 mg./kg., supplemented with 0·1 % in the diet.

Table 1. Plasmodium berghei. *Antagonism of the action of pyrimethamine by p-aminobenzoic acid (PAB) and pteroylglutamic acid (PGA) given subcutaneously (except where indicated) and in the drinking water*

(Parasitaemia is expressed as the average number of parasitized erythrocytes per 1000 erythrocytes, in groups of three mice examined 5–7 days after inoculation.)

| Dose of drug (mg./kg.) | Antagonist | Dose of antagonist (mg./kg.) | Antagonist in drinking water (mg./ml.) | Parasitaemia | |
|---|---|---|---|---|---|
| | | | | Treated mice | Control mice |
| 0·5 | — | — | — | 2 | 80 |
| 0·5 | PAB | 5·0 | — | 0 | 80 |
| 0·5 | PAB | 5·0 | 0·025 | 210 | 80 |
| 0·5 | PAB | 0·5 | 0·025 | 140 | 80 |
| 1·0 | — | — | — | 0 | 90 |
| 1·0 | PAB | 250·0 | — | 0 | 90 |
| 1·0 | PAB | 250·0 | 0·01 | 13 | 90 |
| 0·75 | — | — | — | 0 | 165 |
| 0·75 | PAB | 500·0 | 1·0 | 205 | 165 |
| 0·75 | PAB | 500·0 (oral) | 1·0 | 159 | 165 |
| 0·75 | PAB | 1500·0 (oral) | 3·0 | 190 | 165 |
| 1·25 | — | — | — | 0 | 72 |
| 1·25 | PAB | 500·0 | 1·0 | 55 | 72 |
| 0·25 | — | — | — | 0 | 65 |
| 0·25 | PAB | 1·0 | 0·01 | 75 | 65 |
| 0·5 | — | — | — | 40 | 332 |
| 0·5 | PAB | 0·15 | 0·002 | 290 | 332 |
| 5·0 | PAB | 0·15 | 0·002 | 0 | 332 |
| 0·5 | — | — | — | 2 | 412 |
| 0·5 | PGA | 5·0 | 0·1 | 150 | 412 |
| 0·75 | — | — | — | 0 | 325 |
| 0·75 | PGA | 50·0 | 1·0 | 150 | 325 |

In *P. berghei*, however, the action of pyrimethamine (0·25–1·25 mg./kg.) was inhibited by *p*-aminobenzoic acid and by folic acid when these antagonists were given subcutaneously or orally twice daily and were also dissolved in the drinking water that was continuously available to the mice (Table 1). It was found that a mouse weighing 20 g. drinks about 4 ml. of water in 24 hr., of which approximately 3·5 ml. are taken at night. When antagonists are dissolved in the drinking

water they are therefore taken mostly at night, which balances the subcutaneous or oral injections during the daytime and helps to maintain a constant level of the antagonist in the blood of the treated mice.

Under these conditions, the action of pyrimethamine was inhibited by high doses of *p*-aminobenzoic acid such as 500–1500 mg./kg., together with 1·0–3·0 mg. of *p*-aminobenzoic acid per ml. of drinking water, and also by as low a dose as 0·15–2·0 mg./kg. with 0·002–0·025 mg./ml. of drinking water. When the drinking solution was omitted, however, 5·0 or 250 mg. of *p*-aminobenzoic acid given subcutaneously, twice daily, failed to inhibit the drug.

It was found that *p*-aminobenzoic acid acted as a competitive antagonist because 0·15 mg./kg., with 0·002 mg./ml. of drinking water, antagonized the action of 0·5 mg./kg. of pyrimethamine but did not antagonize the action of ten times this dose of drug in the same experiment. If the antagonism were non-competitive, the above amount of antagonist would have been sufficient to ensure the survival of the plasmodia in any concentration of the drug.

## IV. ANTAGONISM OF SULPHADIAZINE

It has previously been shown with *P. berghei* in mice that a dose of *p*-aminobenzoic of 50 mg./kg. body weight would antagonize 0·5 mg./kg. of sulphadiazine (Thurston, 1950*a*, *b*), and, similarly, that 1000 mg./kg. would antagonize 0·25–25 mg./kg. of sulphadiazine (Mudrow-Reichenow, 1951). Hill (1950) gave 10 mg. sulphadiazine and 40 mg. *p*-aminobenzoic acid per kg. once daily for 3 days and twice on the 4th day. Inhibition was only transient: this was attributed to the rapid excretion of the antagonist and the persistence of the sulphadiazine in the blood.

In the present experiments (Table 2), the action of 0·5 mg. sulphadiazine was completely inhibited by 1·5 mg. *p*-aminobenzoic acid given twice daily, orally or subcutaneously, and by 200 mg. folic acid, given twice daily, orally. No antagonism of sulphadiazine was detected with folinic acid, glycine, histidine, cytosine, uracil, adenine, guanine or riboflavin (each tested in doses of 50 mg./kg.), or with thiamine (100 mg.), D-ribose (200 mg.), thymine (250 mg.), methionine (500 mg.) or nicotinamide (500 mg.).

It was found that *p*-aminobenzoic acid acted as a competitive antagonist because, although 0·5 mg. sulphadiazine was readily antagonized by only 0·15 mg. of *p*-aminobenzoic acid per kg. body weight when given subcutaneously, twice daily, together with 0·002 mg./ml. of drinking water, this dose of *p*-aminobenzoic acid had no inhibitory action when the dose of sulphadiazine was increased to 5·0 mg./kg. Similarly, folic acid, 5·0 mg./kg. given subcutaneously, twice daily, together with 0·1 mg./ml. of drinking water, antagonized the action of 0·5 mg. sulphadiazine but not of 5·0 mg. sulphadiazine.

Competitive antagonism of sulphadiazine by *p*-aminobenzoic acid had also been shown in previous experiments when no additional *p*-aminobenzoic acid was dissolved in the drinking water. The dose of *p*-aminobenzoic acid (1·5 mg./kg.) that would antagonize 0·5 mg. sulphadiazine had no effect on 5·0 mg. sulphadiazine. The dose of *p*-aminobenzoic acid had to be increased to 50 mg./kg. in order to inhibit the action of the higher dose of sulphadiazine. This dose of *p*-aminobenzoic

acid was considerably greater than the dose that Hill (1950) had unsuccessfully tested against a higher dose of sulphadiazine.

Table 2. Plasmodium berghei. *Antagonism of the action of drugs by* p-*aminobenzoic acid* (*PAB*) *and pteroylglutamic acid* (*PGA*) *given subcutaneously and in the drinking water*

(Parasitaemia is expressed as the average number of parasitized erythrocytes per 1000 erythrocytes, in groups of three mice examined 5–7 days after inoculation.)

| Drug | Dose of drug (mg./kg.) | Antagonist | Dose of antagonist (mg./kg.) | Antagonist in drinking water (mg./ml.) | Parasitaemia | |
|---|---|---|---|---|---|---|
| | | | | | Treated mice | Control mice |
| Sulphadiazine | 0·5 | — | — | — | 0 | 165 |
| | 0·5 | PAB | 1·5 | — | 103 | 165 |
| | 0·5 | PGA | 200·0 | — | 150 | 165 |
| | 0·25 | — | — | — | 6 | 165 |
| | 0·25 | PAB | 1·0 | 0·01 | 155 | 165 |
| | 0·5 | — | — | — | 0 | 330 |
| | 0·5 | PAB | 0·15 | 0·002 | 415 | 330 |
| | 5·0 | PAB | 0·15 | 0·002 | 0 | 330 |
| | 0·5 | PGA | 5·0 | 0·1 | 205 | 330 |
| | 5·0 | PGA | 5·0 | 0·1 | 0 | 330 |
| | 0·5 | — | — | — | 0 | 170 |
| | 0·5 | PGA | 50·0 | 1·0 | 105 | 170 |
| Proguanil | 7·5 | — | — | — | 0 | 123 |
| | 7·5 | PAB | 250·0 | 1·0 | 140 | 123 |
| | 7·5 | — | — | — | 4 | 205 |
| | 7·5 | PAB | 0·1 | 0·0015 | 150 | 205 |
| | 7·5 | — | — | — | 3 | 170 |
| | 7·5 | PGA | 50·0 | 1·0 | 30 | 170 |
| Proguanil metabolite CPT | 1·0 | — | — | — | 0 | 70 |
| | 1·0 | PAB | 500·0 | 1·0 | 83 | 70 |
| | 0·5 | — | — | — | 0 | 145 |
| | 0·5 | PAB | 50·0 | 0·5 | 70 | 145 |
| | 1·0 | — | — | — | 1 | 300 |
| | 1·0 | PAB | 0·15 | 0·002 | 15 | 300 |
| | 1·0 | PGA | 5·0 | 0·1 | 35 | 300 |
| Pteridine O/103 | 25·0 | — | — | — | 1 | 65 |
| | 25·0 | PAB | 0·1 | 0·0015 | 60 | 65 |
| | 40·0 | — | — | — | 3 | 205 |
| | 40·0 | PAB | 0·1 | 0·0015 | 150 | 205 |
| | 40·0 | — | — | — | 0 | 170 |
| | 40·0 | PGA | 50·0 | 1·0 | 150 | 170 |

Because of the competitive nature of the antagonism, it was considered that the inhibitory action of a compound would be most readily detected if tested against approximately the minimum effective dose of the drug under investigation. This principle was followed during the present investigation.

## V. ANTAGONISM OF PROGUANIL

Early work indicated that *p*-aminobenzoic acid did not inhibit the action of proguanil against *P. gallinaceum* in the chick (Bishop & McConnachie, 1948; Greenberg, 1949*b*), nor against oocysts of *P. gallinaceum* developing in *Aëdes aegypti* (Terzian *et al.* 1949). Pteroylglutamic acid inhibited the action of proguanil against *Plasmodium gallinaceum* in the chick (Greenberg, 1949*a*), and it was inferred that the drug interferes with the utilization of folic acid rather than its synthesis. Similarly, when proguanil was tested against *Lactobacillus casei in vitro*, in the absence of purines, its action was antagonized by folic acid, guanine, adenine, xanthine and hypoxanthine.

In a preliminary report (Thurston, 1950*b*) it was shown that the action of proguanil against *Plasmodium berghei* could be inhibited by *p*-aminobenzoic acid but not by folic acid. In later experiments, antagonism occurred somewhat inconsistently when the antagonist was given only by mouth, and the experiments were complicated by the increased toxicity to the mice when large doses of *p*-aminobenzoic acid were given at the same time as proguanil. However, when *p*-aminobenzoic acid was also dissolved in the drinking water the action of proguanil, 7·5 mg./kg. twice daily, was inhibited by doses of *p*-aminobenzoic acid down to 0·1 mg./kg., together with 0·0015 mg./ml. of drinking water (Table 2). Similarly, although no antagonism was detected with 200–500 mg. of folic acid given by mouth twice daily, some antagonism was detected with only 50 mg./kg. when the drinking water contained 1·0 mg./ml. The action of the active metabolite of proguanil (CPT) was also inhibited by both *p*-aminobenzoic acid and folic acid when the antagonist was given in the drinking water as well as subcutaneously.

It had seemed as though *P. berghei* differed from *P. gallinaceum* in its dependence on *p*-aminobenzoic acid, but, in a recent paper, Greenberg (1953) has shown that the action of proguanil against *P. gallinaceum* can also be inhibited by *p*-aminobenzoic acid as well as by folic acid and folinic acid, and that thymine, uracil and the nucleic acids show slight tendencies to inhibit the action of proguanil.

## VI. ANTAGONISM OF 2:4-DIAMINOPTERIDINES

The antibacterial activity of several 2:4-diaminopteridines was inhibited by folic acid, and it was suggested that the drugs acted against the utilization rather than the synthesis of folic acid (Daniel, Norris, Scott & Heuser, 1947). Elion & Hitchings (1951) showed with *Lactobacillus casei* that adenine was a non-competitive antagonist, and suggested that the 2:4-diaminopteridines interfered with the synthesis of adenine from folic acid. Collier & Waterhouse (1950, 1952) confirmed that folic acid inhibited the action of the drugs against *Streptococcus faecalis* and found that folinic acid was an even more effective antagonist, whereas neither folic acid nor *p*-aminobenzoic acid antagonized the action of 2:4-diaminopteridines against *Vibrio cholerae*, which synthesizes folic acid. Greenberg (1949*c*) found that folic acid antagonized the action of 2:4-diaminopteridines against *Plasmodium gallinaceum* to the same extent as it antagonized the action of proguanil.

In the present experiments, the action of 0/97, 0/103 and 0/164 against *P. berghei* in the mouse was readily inhibited by *p*-aminobenzoic acid, 50–500 mg./kg. given

orally, twice daily. 0/97 and 0/103 were not antagonized by folic acid, 200 mg./kg. When the antagonist was also dissolved in the drinking water, the action of 0/103 was antagonized by *p*-aminobenzoic acid, 0·1 mg./kg., subcutaneously, with 0·0015 mg./ml. of drinking water, and by folic acid, 50 mg./kg., subcutaneously, with 1·0 mg./ml. of drinking water (Table 2).

When tested against *P. gallinaceum* the action of 0/97 was not inhibited by *p*-aminobenzoic acid, 400 mg./kg. given orally, twice daily, together with 0·1 % of antagonist in the diet, nor by folic acid, 100 mg./kg., orally, twice daily. These doses of *p*-aminobenzoic acid and folic acid had no action on the infection when given alone.

## VII. DISCUSSION

### (1) *Demonstration of antagonism, using* Plasmodium berghei

Similarities between the antimalarial action of sulphonamides, proguanil, pyrimethamine and 2:4-diaminopteridines have previously been found during studies on morphological changes and cross-resistance (Thurston, 1953*a*, *b*). The antagonism of all of these drugs by *p*-aminobenzoic acid and by folic acid indicates another similarity in their mode of action.

Antagonism was in most cases detected only when the antagonist was given continuously, by being dissolved in the drinking water as well as being injected subcutaneously. Antagonism was always observed when large doses of *p*-aminobenzoic acid were given but the very low doses that have been recorded in Tables 1 and 2 were inhibitory only at the minimum effective dose of the antimalarial drug, and the results were greatly influenced by the degree of infection in the mice during treatment. The minimum effective dose of each of the antimalarial drugs was apparently antagonized by approximately the same minimum dose of *p*-aminobenzoic acid. The antagonism of pyrimethamine and sulphadiazine was competitive. The inhibitory doses of folic acid were higher than those of *p*-aminobenzoic acid.

No inhibition was detected when several amino-acids and nucleic acid derivatives were tested against pyrimethamine and sulphadiazine, the compounds being given by mouth twice daily. As it is not possible to demonstrate the antagonism of pyrimethamine by *p*-aminobenzoic acid by this schedule, but only if the antagonist is dissolved in the drinking water, it may be that in a similar manner the amino-acids and nucleic acid derivatives would be inhibitory if they were given in the drinking water as well as being injected twice daily. However, low doses of *p*-aminobenzoic acid inhibit the action of sulphadiazine when given twice daily, without there being any in the drinking water. Therefore, should any antagonism be detected with the amino-acids and nucleic acid derivatives when given by a different dose schedule, it can still be concluded that these compounds are less effective than *p*-aminobenzoic acid in antagonizing sulphadiazine.

### (2) *Comparison of* P. berghei *with other micro-organisms*

Until recent work by Greenberg (1953) demonstrated slight antagonism by *p*-aminobenzoic acid of the action of proguanil against *P. gallinaceum*, it had seemed as though there were a fundamental difference between *P. berghei* and

*P. gallinaceum* in their dependence upon *p*-aminobenzoic acid. It is always difficult in experiments *in vivo* to assess the amount of any given compound that is available to the parasite before it is metabolized by the host, and it was thought that the different results might depend more on differences between the hosts than between the parasites. However, there does not appear to be any obvious difference between the metabolic fate of *p*-aminobenzoic acid in mice and in chicks, nor between the synthesis of folic acid by the intestinal flora of the two hosts.

Although 2:4-diaminopteridines were usually considered to be folic acid antagonists, Daniel & Norris (1947) and Daniel *et al.* (1947) found that the action of these drugs against *Lactobacillus arabinosus* could be antagonized by *p*-aminobenzoic acid, as *L. arabinosus* requires *p*-aminobenzoic acid to synthesize folic acid. In a similar way, *Plasmodium berghei* is probably more readily able to synthesize folic acid from *p*-aminobenzoic acid than is *P. gallinaceum*, and so tends to resemble *P. knowlesi* which, when it was maintained *in vitro* by Anfinsen, Geiman, McKee, Ormsbee & Ball (1946) was found to require *p*-aminobenzoic acid and to be unaffected by folic acid.

### (3) *The sensitivity of* P. berghei, P. gallinaceum, P. knowlesi *and* P. cynomolgi *to the action of drugs*

Besides its postulated dependence on *p*-aminobenzoic acid, *P. berghei* resembles *P. knowlesi* in its extreme sensitivity to sulphonamides, whereas *P. gallinaceum* and *P. cynomolgi* are less sensitive to these drugs. As the mode of action of proguanil, pyrimethamine and the 2:4-diaminopteridines has been shown to be similar to that of the sulphonamides, it was thought that *P. berghei* and *P. knowlesi* should similarly be more sensitive to these drugs than were *P. gallinaceum* and *P. cynomolgi*. Table 3 shows the minimum effective dose range of all these drugs against the four species of *Plasmodium*. In most cases two doses are quoted for each drug, representing the lowest and the highest doses reported in the literature. The differences in these dose levels probably depend chiefly upon differences in the conception of the minimum effective dose, for example, Rollo uses a reduction of parasitaemia by only one-half whereas the present author requires a reduction of one-fiftieth. Schmidt & Genther (1953) showed that the range of action of pyrimethamine against *P. cynomolgi* was very wide, for although a dose as low as 0·0024 mg./kg. had some effect on the parasites, as much as 0·6 mg./kg. was necessary to sterilize the infection.

From Table 3 it can be seen that there is no consistent pattern of activity with these drugs. Pyrimethamine is as active against *P. gallinaceum* and *P. cynomolgi*, which are only slightly sensitive to the action of the sulphonamides, as it is against *P. berghei* and *P. knowlesi*, which are extremely sensitive to the sulphonamides. Proguanil and pyrimethamine are less active against *P. berghei* than against *P. knowlesi*, although these two species are equally sensitive to sulphadiazine. *P. berghei* resembles *P. gallinaceum* in being moderately sensitive to the action of proguanil and more sensitive to the proguanil metabolite (CPT), whereas *P. cynomolgi* is very sensitive to the action of proguanil and yet is less sensitive to CPT. *P. gallinaceum* is generally more sensitive to the action of the pteridines than is

*P. berghei*, but there is no correlation between the degree of activity of the different pteridines against the two species. For example, 0/120/II, 2:4-diamino-1′-methylindolo-2′:3′-6:7-pteridine, was inactive against both *P. gallinaceum* and *P. berghei* while its 1′-ethylindolo-analogue was inactive against *P. gallinaceum* but showed considerable activity against *P. berghei*.

Table 3. *The minimum effective dose of drugs, expressed as mg. per kg. per dose, against four species of* Plasmodium

(When no reference is given, the result was obtained by the author. The 2:4-diaminopteridines have the following substituents in the 6:7 positions: 0/63 -diphenyl, 0/79 -camphano, 0/103 -*di*nhexyl, 0/120/II -1′-methylindolo, 0/129 -di*iso*propyl, and 0/164 -1′-ethylindolo. b.i.d.= drug given twice daily.)

| Drug | *P. gallinaceum* | *P. berghei* | *P. knowlesi* | *P. cynomolgi* |
|---|---|---|---|---|
| Sulphadiazine | 30·0 b.i.d. (Greenberg, Boyd & Josephson, 1948) | 0·05 b.i.d. (Rollo, 1951) | 0·05–0·125 (Singh, Ray, Basu & Nair, 1952) | 125 (Hawking & Thurston, 1951) |
| | 62·5 b.i.d. (Bishop & McConnachie, 1950) | 0·25 | — | — |
| Proguanil | 2·0 b.i.d. (Greenberg & Richeson, 1951) | 5·4 b.i.d. (Rollo, 1951) | 0·1 (Singh *et al.* 1952) | 0·0188 (Schmidt, Genther, Fradkin & Squires, 1949) |
| | 5·0 b.i.d. (Davey, 1946) | 12·0 | — | 0·075 (Schmidt, Loo, Fradkin & Hughes, 1952) |
| Proguanil metabolite CPT | 10 × proguanil, i.e. 0·5 b.i.d. (Crowther & Levi, 1953; Schmidt *et al.* 1952) | 0·25 | — | 0·3 (Schmidt *et al.* 1952) |
| Pyrimethamine | 0·03 b.i.d. (Rollo, 1951) | 0·02 b.i.d. (Rollo, 1951) | 0·005 (Singh *et al.* 1952) | 0·0024–0·6 (Schmidt & Genther, 1953) |
| | 0·2 b.i.d. | 0·5 | — | — |
| Pteridines 0/63 | 16·0 b.i.d. (Greenberg & Richeson, 1951) | 250·0 | — | — |
| | 32·0 b.i.d. (McConnachie, 1953) | — | — | — |
| 0/97 | < 2·5 b.i.d. | 25·0 | — | — |
| 0/103 | 40·0 b.i.d. | 25·0 | — | — |
| 0/120/II | > 500·0 b.i.d. (McConnachie, 1953) | > 500·0 | — | — |
| 0/129 | 2·5 b.i.d. | — | — | — |
| | 3·15 b.i.d. (McConnachie, 1953) | 25·0 | — | — |
| 0/164 | > 40·0 b.i.d. | 25·0 | — | — |

The differences in sensitivity are probably related more to differences in the metabolism of the parasites and to differences in the mode of action of the drugs than to differences in the absorption of the drugs in the different hosts, because *P. knowlesi* and *P. cynomolgi* are both parasitic in the rhesus monkey and yet differ greatly in their sensitivity to drugs. Likewise, *P. berghei* is 300 times as sensitive to the action of sulphadiazine as is *P. gallinaceum*, although the blood level of sulphadiazine in mice is only twice as high as in chicks (Hill, 1950).

## VIII. SUMMARY

The action of pyrimethamine, sulphadiazine, proguanil and its active metabolite CPT, and 2:4-diaminopteridines against infections of *Plasmodium berghei* in mice was antagonized by *p*-aminobenzoic acid and by pteroylglutamic acid. Antagonism was in some instances detected only when *p*-aminobenzoic acid was given in solution in the drinking water as well as being injected subcutaneously. No antagonism was detected with a number of amino acids and nucleic acid derivatives.

As all of the above group of drugs can be antagonized by *p*-aminobenzoic acid and by pteroylglutamic acid, it would seem that they are alike in their mode of action. There must, however, be some differences between the mode of action or absorption of these drugs because species of *Plasmodium* that are very sensitive to the action of one of these drugs are frequently not very sensitive to the action of others.

As *P. berghei* is dependent on *p*-aminobenzoic acid, it is suggested that it can utilize this compound in the synthesis of pteroylglutamic acid to a greater extent than can *P. gallinaceum*, and that it resembles *P. knowlesi* more than other species of *Plasmodium*.

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# NOTES UPON THE BIOLOGY AND MORPHOLOGY OF THE IMMATURE STAGES OF *NEOTTIOPHILUM PRAEUSTUM* (MEIGEN, 1826) (DIPTERA: NEOTTIOPHILIDAE) PARASITIC ON BIRDS

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(With 11 Figures in the Text)

Although the acalypterate fly, *Neottiophilum praeustum* (Meigen, 1826) has been bred from nests of birds repeatedly and the third-instar larvae and pupae have been found in nests of a number of different species of birds, the full life history and the morphology of the early larval stages of this insect have not been recorded. In the summer of 1947 I found several nests of finches with heavy infestations of the larval stages of this fly. This material enabled me to study the morphology of the second-instar larva and to make some observations on the mode of life of the second and third larval stages.

The species of birds from the nests of which *N. praeustum* has been recorded have been listed by Keilin (1924), by Czerny (1930) and by Séguy (1950). Omitting some records in which the species of host was not determined, the birds known to serve as hosts up to date are:

(1) Greenfinch, *Chloris chloris chloris* (L.).—Patton (in Keilin, 1924), Czerny (1930), Séguy (1950).

(2) Chaffinch, *Fringilla coelebs coelebs* L.—Bezzi (1922), Patton (in Keilin, 1924), Czerny (1930).

(3) Linnet, *Carduelis cannabina cannabina* (L.).—Keilin (1924), Czerny (1930), Séguy (1950).

(4) Sparrow, *Passer domesticus domesticus* (L.).—Bezzi (1922), Czerny (1930), Séguy (1950).

(5) Blackcap, *Sylvia atricapilla atricapilla* (L.).—Keilin (1924), Czerny (1930), Séguy (1950).

(6) Nightingale, *Luscinia megarhyncha megarhyncha* Brehm.—Séguy (1934, 1950).

(7) Wren, *Troglodytes troglodytes troglodytes* L.—E. A. Armstrong (1953), E. B. Basden (in Armstrong, 1953).

(8) Tree-creeper, *Certhia familiaris familiaris* L.—Hesse (1923), Czerny (1930), Séguy (1950).

(9) Blackbird, *Turdus merula merula* L.—Collin (in Keilin, 1924), Hesse (1923), Czerny (1930), Séguy (1950).

(10) Thrush, *Turdus ericetorum ericetorum* Turton.—Collin (in Keilin, 1924), Czerny (1930), Séguy (1950).

(11) Sparrowhawk, *Accipiter nisus nisus* (L.).—Dorn (cited by Hesse, 1925), Czerny (1930).

I have once found larvae of this fly in a thrushs' nest in 1938; and the material upon which the present work is based was from nests of the linnet and the greenfinch. Although nests of thrushes and blackbirds containing nestlings were found in the same hedges, close to the infected nests of linnets and greenfinches in 1947, none of them was infested with *Neottiophilum praeustum*. With reference to *N. praeustum*, Collin (cited by Keilin, 1924) states that 'I have personally bred it freely from the nests of thrushes and blackbirds', whereas Patton (cited by Keilin, 1924) says '. . . but we have never found them in the nests of Thrushes and Blackbirds, though we have searched for them on many occasions. It is very common in my garden but only in the nests noted' (i.e. chaffinches' and greenfinches'). These observations indicate that there may be marked 'host specificity' in *N. praeustum*, and that the hosts of preference may not be the same in different regions, but many more observations would be needed to prove this.

## BIOLOGY OF THE LARVAE OF *NEOTTIOPHILUM PRAEUSTUM*

A linnets' nest provided material for most of the observations upon the biology of the larvae recorded in this paper. When found (27 May 1947) the nest contained four nestlings about 1 week old. Two of the nestlings were dead and, when removed from the nest, they proved to be infected with larvae of *N. praeustum*. Some of the larvae were attached by their mouthparts to the skin, but some had bored holes through the skin in the folds between the thighs and the abdomen and had penetrated into the body of the nestlings. The larvae attached to the nestlings had the alimentary canal full of blood. Two days later (29 May 1947) the nest was deserted and only one dead nestling remained in it; the other was found dead and decomposing in the hedge beneath the nest. The nest containing the dead bird was removed to the laboratory and carefully examined. The nestling had partly decomposed and a number of *N. praeustum* larvae had entered the abdominal cavity and were found mostly in the vicinity of the liver. Eleven large and three small third-instar larvae were collected from the abdominal cavity. Many *N. praeustum* larvae were also present in the nest. Altogether, the following larvae were collected from this one nest (no. 81): on, or in, dead nestlings, 28 third-instar larvae; in the nest, 16 second-instar and 82 third-instar larvae. Thus, the total number of larvae collected from this nest was 16 second instar and 100 third instar.

Another linnets' nest (no. 82) containing three dead nestlings was found in the same hedge on 3 June 1947, and from it *N. praeustum* larvae were also collected as follows: in the abdominal cavity of nestlings, 4 third instar; in the nest, 100 third instar. Total, 104 third-instar larvae.

In an empty greenfinches' nest (no. 84) in a nearby apple tree, on 19 June, six third-instar larvae of *N. praeustum* were found. There was a thrushes' nest containing nestlings in the same hedge as the heavily infested linnets' nest (no. 81) and only about 2 yards away, but no *N. praeustum* could be found in it after the nestlings had flown. It is also of interest that later in the year, in August, six nests, probably five linnets' and one greenfinches' from the same hedge as nos. 81 and 82, or within a short distance of it, were examined but no *N. praeustum*

larvae were found. This suggests that there is only one generation of this fly in the season.

Patton (cited by Keilin, 1924) stated 'I first collected the larvae of *N. praeustum* in the summer of 1919 from the nest of a Greenfinch, I then noted that they contained the blood of the nestlings', and Keilin (1924), on morphological grounds, concluded that the larvae of *N. praeustum* live as intermittent blood-sucking parasites. Hitherto no direct observations on the feeding habits of the larvae had been recorded.

In the present observations larvae engorged with blood were found still affixed to the nestlings by their mouthparts and there can be no doubt that they were definitely blood suckers. The entry of larvae into the bodies of dead nestlings and their feeding upon abdominal viscera has not been recorded previously although Armstrong (1953) states that Basden found 38 *N. praeustum* larvae in a wrens' nest containing two dead nestlings which had presumably succumbed to the attacks of the larvae. Some experiments were made upon the feeding habits of larvae collected from nests nos. 81 and 82. When young third-instar larvae were put on fresh horse meat they wandered away from it and made no attempt to feed. When such larvae were placed upon a freshly killed young chick of the domestic fowl, a small number of them became affixed to the skin and ingested blood. In one experiment, when 25 larvae were put on a dead chick and left overnight, one buried itself in the subcutaneous tissue of the chick, and, in addition, there were three holes made in the skin of the chick and several larvae were attached to it by their mouthparts. When young third-instar larvae were placed within the abdomen of a freshly killed chick, they did not stay there but crawled away from the body. These experiments, and the fact that larvae were found attached to, and within the abdominal cavity of, dead nestlings indicated that the larvae might be able to continue to live and develop upon dead birds once they could no longer suck blood of living hosts. However, continued observation of larvae which had fed upon dead birds showed that they became abnormally distended and died rapidly, usually within a day or two, and none of them survived to pupate. Therefore, feeding upon dead birds would appear to be aberrant behaviour and not part of the normal life cycle.

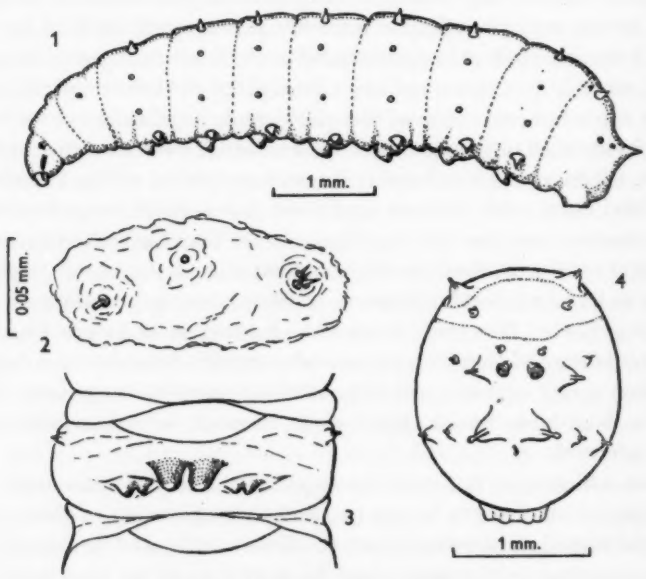
The larvae which were not used for experiments or dissection were placed in moist sawdust to allow them to pupate; but although many of them must have been fully developed only seven pupated. Hesse (1923) and Keilin (1924) found that larvae remained in the same stage for a long time, up to 4 months, before pupation which did not occur until autumn. In my material also, although apparently fully grown larvae were collected in late May and early June, puparia were not formed until between 16 October and 12 November. The small number of pupae obtained suggests that sawdust may not be a very favourable medium for pupation for this species, although it is very good for many diptera such as *Calliphora*. Parasitic diptera may find sawdust unsatisfactory for pupation as Brumpt (1934) found that *Lucilia bufonivora*, parasitic in toads, pupated well in soil, whereas in sawdust nearly 50 % of the larvae failed to pupate.

The seven *Neottiophilum praeustum* pupae were kept at room temperature until

the following spring, but no flies emerged from them although flies were obtained by Hesse (1923, 1925) between 2 February and 25 April, and by Keilin (1924) between the end of March and the end of April from pupae kept at laboratory temperature throughout the winter. When these seven puparia were dissected, fully formed flies were found within them so that it was a failure of emergence and not a failure of development within the puparia. Possibly the sawdust became too dry during the long period at room temperature and the flies were not vigorous enough to rupture the puparia. Nevertheless, they provided material upon which the occurrence of a fourth, intrapuparial, moult could be determined.

## SECOND-INSTAR LARVA

One larva was found on the point of undergoing the second moult and clearly showing the morphological features of the third instar beneath the second-instar cuticle. This made it certain that the second-instar larvae found in the nest were part of the life cycle of *N. praeustum*.




Figs. 1–4. *Neottiophilum praeustum*.

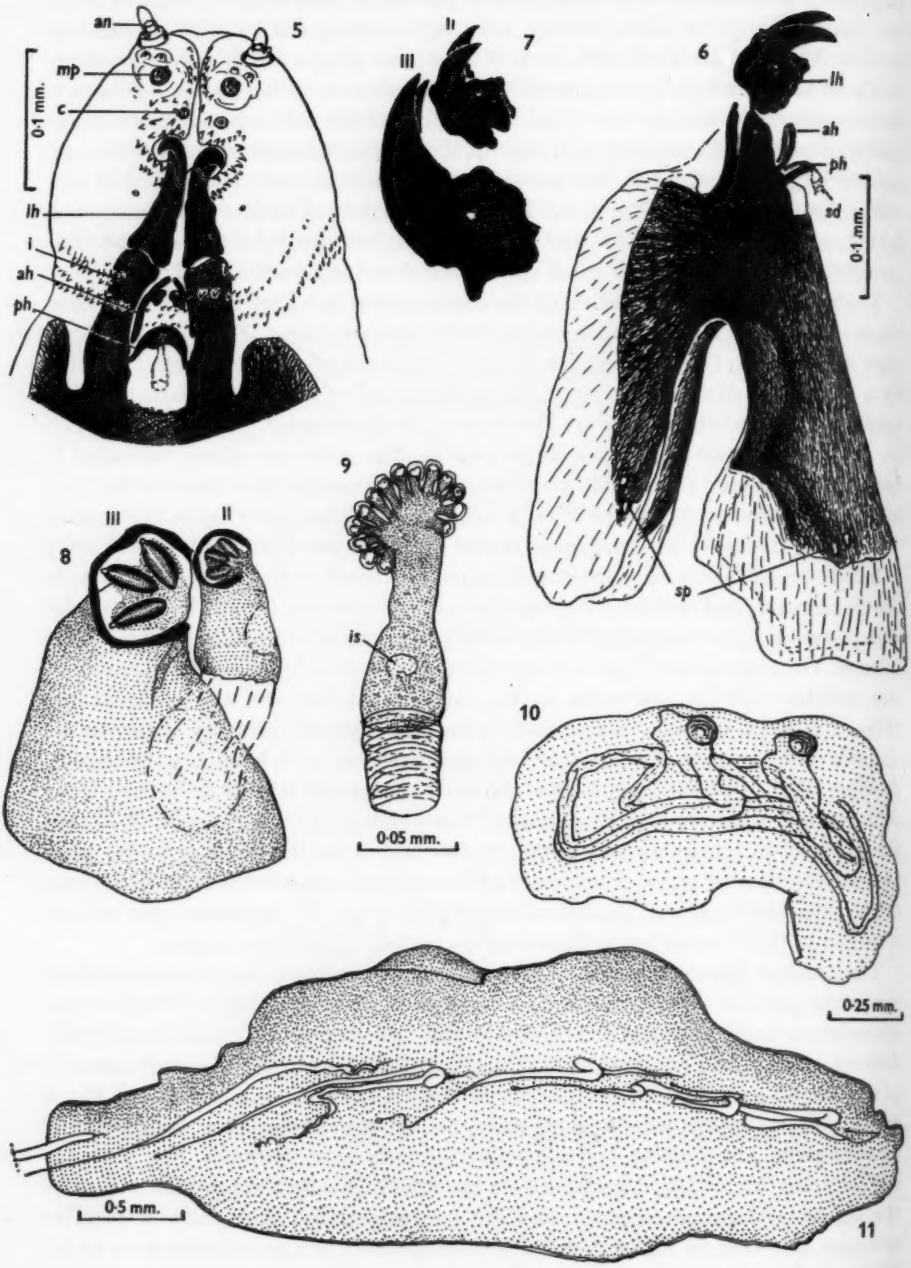
Fig. 1. Second-instar larva, lateral view. Fig. 2. Second-instar larva, sensory structures on plaque on right side of ventral surface of third thoracic segment. Fig. 3. Second-instar larva, ventral surface third abdominal segment (same scale as Fig. 4). Fig. 4. Second-instar larva, posterior aspect.

The second-instar larva (Fig. 1) is about 8 mm. long and is brownish in colour and more cylindrical than the third-instar larva. Rows of small spines surround the anterior margin of each segment. The three thoracic segments are devoid of

papillae or protuberances. On the ventral surface of each thoracic segment there are paired groups of three sensory hairs representing the vestiges of the legs (Keilin, 1915). Associated with each of these hair groups wrinkles in the cuticle mark off elongated plaques upon which are borne, in addition to the leg-hairs, a sensory pit and a sensory rod (Fig. 2). The first abdominal segment has a single pair of dorso-lateral papillae; and each of the abdominal segments 2 to 7 has one pair of large dorso-lateral, two pairs of smaller lateral, and one pair of double ventro-lateral papillae (Figs. 1, 3). The ventral surface of these segments is much folded, and on each there is a pair of contiguous, backwardly directed, extensible protuberances, covered with small spines, which aid in locomotion (Fig. 3).

The head and buccal armature of the second-instar larva are much smaller than those of the third instar but otherwise closely resemble them (Figs. 5–7, and compare with Keilin, 1924, pl. IX, figs. 2 and 6–8). The head is divided into two lobes by a ventral longitudinal groove at the posterior end of which lies the mouth. The antennae and maxillary palps are like those of the third instar, but sensory organ C on each side consists of only a single papilla. The cuticular spines, arranged in broken rows around the mouth, are relatively much smaller than those in the third instar and there are no groups of large spines forming 'scrapers' as in that instar. The lateral hooks of the bucco-pharyngeal armature are equal and have sharply pointed curved blades. The intermediate piece is fused to the basal sclerite, as in the third instar, and ventrally there are two arc-like sclerites, formed by the anterior and posterior hypopharyngeal rods, uniting the lateral bars of the intermediate sclerite. The anterior arc (Figs. 5, 6, *ah*) is formed of two curved rods which approach one another but do not unite in the mid-ventral line and the posterior arc (Figs. 5, 6, *ph*) is continuous and receives the salivary duct ventrally. Between the anterior and posterior arcs there are two small sclerites, each bearing a few sensory papillae (Fig. 5). The lateral plates of the basal pharyngeal sclerite differ from those of the third instar in having long anterior dorsal rods projecting forwards from near the union of intermediate and basal sclerites and in having on each lateral plate two pairs of sensory pores, one dorsal and one ventral, towards the posterior border of the sclerotized parts of the lateral wings (Fig. 6, *sp*). These sensory pits are not seen in the third-instar larva. There are no ventral pharyngeal ridges.

The anterior spiracles, borne on the first thoracic segment, have about fourteen spiracular papillae each, and the internal scar is clearly shown (Fig. 9). The posterior spiracles are small and each has three straight, radiating spiracular clefts and well-defined internal and external scars. The similarity between the posterior spiracles of the second and third instars, except for the difference in size, is shown in Fig. 8 which is drawn from a second-instar larva just about to undergo the second ecdysis in which the third-instar spiracles have already been formed. The posterior spiracles are borne close together above the middle line on the posterior aspect of the eighth abdominal segment, and are surrounded by eight pairs of papillae arranged as shown in Fig. 4. Ventrally this segment has a protuberance on which the anus opens.




Figs. 5–11. *Neottiophilum praeustum.*

## THE FOURTH, OR PREPUPAL MOULT, IN *NEOTTIOPHILUM PRAEUSTUM*

Snodgrass (1924) described a prepupal moult in the acalypterate fly *Rhagoletis pomonella* Walsh, and later Fraenkel (1938) described a partial prepupal moult in several calypterate diptera including *Calliphora*. Recently, Tate (1953) has shown that there is a complete prepupal moult in *C. erythrocephala*, although the anterior region of the prepupal cuticle is extremely delicate and difficult to reveal.

To extract the dead flies from the puparia of *Neottiophilum praeustum*, the puparia were softened by soaking overnight in cold 5 % KOH. When the flies were extracted a thin pellicle was easily separated from the puparium, and it proved to be a definite prepupal cuticle, closely resembling that described by Snodgrass for *Rhagoletis pomonella*. This cuticle is much stouter than that of *Calliphora erythrocephala* and has a finely mamillated outer surface as in *Rhagoletis*. Applied to its inner surface in the postero-dorsal region are the folded posterior tracheal trunks (Fig. 10) which must have been extracted in relays by swaying of the pupal abdomen as has been described for *Calliphora erythrocephala* (Tate, 1953). The cast tracheae from the lateral spiracles are fixed to the inner surface of the fourth cuticle in the mid-lateral line (Fig. 11). It was not possible to extract an unbroken fourth cuticle and, consequently, the number and exact arrangement of the lateral spiracles and associated tracheae could not be determined. Snodgrass (1924) described eight abdominal spiracles in *Rhagoletis*; but in the prepupal cuticle of *Neottiophilum* I could see only two thoracic and seven abdominal spiracles, which is the same number as I have found in the prepupa of *Calliphora erythrocephala*.

## DISCUSSION

There can be no doubt that the second- and third-larval instars of *Neottiophilum praeustum* are true parasites and live by sucking the blood of birds. Probably their attacks are confined to the nestlings as they have not been described from adult birds. It is difficult to understand how they could escape being destroyed by pecking or preening if they became attached to the parent birds, and there would also be the danger of their being carried away from the nests and dropped at random before they were fully grown. Therefore, the larval life must be closely correlated with the nesting habits of the hosts, and larval feeding must be

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Fig. 5. Second-instar larva, anterior region, ventral. Fig. 6. Second-instar larva, buccopharyngeal armature, lateral. Fig. 7. Developing third-instar lateral hooks behind the lateral hooks of a second-instar larva about to moult (same scale as Fig. 6). Fig. 8. Overlapping second- and third-instar posterior spiracles in a second-instar larva about to moult (same scale as Fig. 6). Fig. 9. Second-instar larva, prothoracic spiracle. Fig. 10. Postero-dorsal part of the moulted fourth, or prepupal, cuticle. External view showing cast posterior tracheal trunks of prepupa folded and stuck to the inner surface of the cuticle. Fig. 11. Internal view of right side of fourth (prepupal) cuticle. *Key*: *ah*, anterior hypopharyngeal rod; *an*, antenna; *c*, sensory organ C; *i*, intermediate sclerite; *is*, internal scar; *lh*, lateral hooks; *mp*, maxillary palp; *ph*, posterior pharyngeal rod; *sd*, salivary duct. II, lateral hook or spiracle of second-instar larva; III, developing lateral hook or spiracle of third-instar larva.

completed during the nestling period as once the brood has flown opportunities for further feeding must be rare, unless the immature larvae survive in the nest and again attack nestlings should a second brood be raised in the same nest. The present observations, and those recorded by Basden (in Armstrong, 1953), show that if the larvae are numerous their attacks kill the nestlings. Once the nestlings are dead, although the larvae may continue to feed on the bodies, apparently they cannot survive without fresh blood and the ingestion of food from the corpses results in the death of the larvae within a few days. Probably bacterial decomposition in the dead food makes it unsuitable for assimilation by the larvae.

The prepupal cuticle in *N. praeustum* is relatively more developed than in *Calliphora erythrocephala*, and it can be dissected out of the puparium much more easily. It resembles that described by Snodgrass (1924) in *Rhagoletis pomonella*, and probably the prepupal stage in the acalypterates is less reduced and the cast cuticle therefore more distinct, than in calypterates such as *Calliphora* in which this stage is of very short duration and the cuticle is a very delicate, almost vestigial, membrane. This suggests that the prepupal stage is a primitive character in the cyclorrhaphous Diptera and becomes progressively reduced in importance in passing from the lower to the higher families.

## SUMMARY

1. The feeding habits of second- and third-instar larvae of *Neottiophilum praeustum* have been observed and show that this species is a true parasite of birds and feeds by sucking the blood of nestlings.

2. If they are too numerous the larvae may kill the nestlings. Although they will continue to feed upon dead birds, and even penetrate into the viscera, such food is unsuitable for the development of the larvae and they become greatly distended and die within a few days.

3. The morphology of the hitherto unknown second-instar larva is described and is compared with that of the third instar.

4. Within the puparium of *Neottiophilum praeustum* there is a fourth moult resulting in the formation of a cast prepupal cuticle which resembles that described by Snodgrass in *Rhagoletis pomonella* and is much better developed than the prepupal cuticle in *Calliphora erythrocephala*.

5. The better development of the prepupal cuticle in the acalypterates than in calypterates indicates that the presence of a prepupal stage in the cyclorrhaphous Diptera is a primitive character and is progressively reduced until in the higher families it is almost vestigial.

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# THE EFFECT OF SULPHADIAZINE, PROGUANIL AND 2:4-DIAMINO-6:7-DI*ISO*PROPYLPTERIDINE UPON GAMETOCYTE PRODUCTION IN *PLASMODIUM GALLINACEUM* (BRUMPT, 1935)

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## INTRODUCTION

It was noted by Bishop & Birkett (1948) that, in the majority of chicks infected with the proguanil-resistant† strain of *Plasmodium gallinaceum*, the proportion of gametocytes to asexual parasites was greater than in the untreated parent strain, though both strains were maintained by the same method of passage. Moreover, whereas the rate and intensity of infection produced in mosquitoes fed upon birds infected with the untreated parent strain was low, in mosquitoes fed upon birds infected with the proguanil-resistant strain it was high. A similar high rate of infectivity and intensity of infection was produced in mosquitoes by the sulphadiazine-resistant strain (Bishop & McConnachie, 1950).

In their investigation of the therapeutic action of proguanil upon malignant tertian malaria, Maegraith, Adams, King, Townshend, Davey & Havard (1945) found that whereas the drug cleared the asexual forms from the blood, the gametocytes did not appear to be affected by its action. Their observation was confirmed by Fairley (1946) who found that, in therapeutic doses, proguanil affects neither the microscopic appearance nor the numbers of gametocytes already formed, but it has an indirect action upon them in that it terminates the primary trophozoite wave prematurely, soon after the appearance of asexual parasites in the blood.

Observations by other workers upon human malaria suggest that an increase in the numbers of gametocytes produced may occur in patients treated with proguanil or sulphonamides. Thus Mackerras & Ercole (1948) observed an increase in the numbers produced on the 2nd and 3rd day of treatment with proguanil, in patients infected with *P. vivax*, which was more marked than in those treated with quinine, atebrin or plasmoquine. Shute & Maryon (1951) found that gametocyte production was greater in cases in which the primary attack had been aborted by treatment with antimalarial drugs. In twelve cases treated with proguanil, gametocytes were numerous during the first recrudescence. Although these authors believe that proguanil may have stimulated the development of gametocytes, they consider that such an action must be indirect, and that any drug which fails to cure the primary attack allows gametocyte production to proceed unchecked. They also observed that in patients infected with *P. falciparum* and

* Member of the Scientific Staff of the Medical Research Council.
† Proguanil = paludrine, *N*-*p*-chlorophenyl-$N^5$-*iso*propylbiguanide.

treated with sulphamethazine, gametocytes appeared in enormous numbers. This observation recalls a previous one made by Findlay, Maegraith, Markson & Holden (1946), who described the appearance of showers of gametocytes in the peripheral blood, both during and after treatment, of patients treated with sulphamethazine, whereas the numbers produced in patients treated with quinine or mepacrine were not large. Ramakrishnan, Young, Jeffery, Burgess & McLendon (1952) found that in some patients infected with *P. falciparum* and treated with proguanil, the density of gametocytes was greater than in the control patients, but they were of the opinion that more proof was needed before it could be concluded that proguanil brought about an increase in gametocyte density.

In view of these observations, and those of my co-workers and myself upon proguanil- and sulphadiazine-resistant strains of *P. gallinaceum*, a series of experiments was performed to try to discover if sulphadiazine or proguanil had any direct effect upon the numbers of gametocytes produced. It was observed that in a strain of *P. gallinaceum* in which resistance to 2:4-diamino-6:7-di*iso*propylpteridine was being produced by treatment with that drug, large numbers of gametocytes were present. The effect of this drug upon gametocyte production, therefore, is also included in this study.

## METHODS

The strains of *P. gallinaceum* used in these experiments were all substrains of the parent strain from which the drug-resistant strains had been derived. This strain had, at no time in its history, been in contact with any drug. Since 1945, it had been maintained solely by blood inoculation. With the exception of several periods of a few weeks during which it was passaged intramuscularly at intervals of 5 or more days, it has been maintained by intravenous injection at intervals of 2–4 days.

The drugs were given orally, by catheter tube into the stomach, proguanil being given as a solution of the hydrochloride, and 2:4-diamino-6:7-di*iso*propylpteridine and sulphadiazine as suspensions in 10% gum acacia.

The numbers of gametocytes produced in drug-treated strains were compared with those produced by the parent strain by the method described previously for assessing drug-resistance (Bishop & Birkett, 1948). In these tests groups of five or six chicks of the domestic fowl, 4–10 days old, were inoculated intravenously with 50 million parasitized erythrocytes of the drug-treated strain per bird, and the resulting infections compared with those produced by similar inocula of the parent strain in untreated birds. The intensity of infection was assessed as the number of parasitized cells per 500 erythrocytes, and the gametocyte rate as the number of fully grown gametocytes of both sexes per 10,000 erythrocytes. Infections lighter than twenty parasitized erythrocytes per 500 were not included as it was felt that they could be of little comparative value. Only gametocytes which practically filled the red cells, and in which the pigment grains were scattered, were counted. Blood films were made immediately after inoculation, in order to assess the numbers of gametocytes present in each bird at the beginning of the experiment. Previous experience had shown that, with the parent strain, maximum parasitaemia was attained, in the majority of young chicks 4–10 days old, on the 4th-5th days

following the intravenous inoculation of 50 million parasitized red cells, and that death due to the infection might occur on or after the 5th day. The blood films on which the final counts were based, therefore, were made between 9·30 and 10.30 a.m. on the 4th day following inoculation, but in one experiment films were also made between 4·0 and 5·0 p.m. on that day, and, in two experiments, between 9.30 and 10.30 a.m. on the following day.

## EXPERIMENTAL

An apparent increase in the proportion of gametocytes to asexual parasites was observed in strains of *P. gallinaceum* maintained in a patent state by passage at intervals of 2–5 days through birds undergoing treatment with sulphadiazine, proguanil or 2:4-diamino-6:7-di*iso*propylpteridine. This increase was not, however, observed throughout the whole period during which the strains were undergoing treatment with the drugs. In producing drug-resistant strains of *P. gallinaceum* a substrain of the parent strain was passaged through chicks which, in the initial passages, were treated once or twice daily with doses of drug so small that they had little effect upon the intensity of the infections produced. The dose was gradually increased through later passages as resistance developed, care being taken that any increase in dosage was not so great that it inhibited the growth of the parasites completely. Each increase in the dose of drug to which the strain was exposed was followed by a period of readjustment in the parasites. The intensity of infection at first decreased, and many parasites, particularly schizonts, became abnormal in appearance. The nuclei appeared to be more readily affected than the cytoplasm, parasites of maximal size being observed which contained a mass of undivided nuclear material which stained poorly with the Giemsa stain, or which contained less than the normal number of merozoites. As the strain became resistant to the drug the division rate, expressed as the intensity of infection, increased and the appearance of the parasites returned to normal. Increases in the proportion of gametocytes to asexual parasites were particularly evident when these readjustments to increased dosage were almost complete. Their numbers tended to fall again once the strain had become fully resistant to the particular dose of drug.

In strains in which drug-resistance was being induced, the inocula used varied from 0·1 to 1·0 ml. of heparinized, infected blood, since it was believed that resistance was more likely to develop if the population of parasites exposed to the action of the drug was large. Such inocula were sufficient to produce a patent infection in young birds immediately, provided that the infection of the donor bird was heavy. Since it was known that proguanil and sulphonamides are schizonticidal and not gametocidal, and since the survival time of gametocytes of *P. gallinaceum* was not known, it was at first thought that the increase in numbers of gametocytes might be due to an accumulation of these forms as a result of the large inocula and the schizonticidal action of the drug. To prove if this were the case the following experiments were performed.

A substrain, SD.A, of the parent strain was passaged at intervals of 2–4 days through chicks treated with 0·2 mg. of sulphadiazine twice daily for a period of

10 days, followed by passage at similar intervals through birds treated with 0·3 mg. of sulphadiazine twice daily.* These doses were sufficient, in the early passages, to reduce the intensity of infections produced by inocula of 0·1–0·25 ml. of heparinized blood, but not to suppress the infections completely. A second substrain, strain G, of the parent strain was maintained in a similar manner, the only difference being that it was passed through normal birds and not through birds receiving drug. After a total of fifteen passages over a period of 41 days, the numbers of gametocytes produced by strain SD.A in untreated birds and in birds treated with 0·1, 0·2, or 0·3 mg. doses of sulphadiazine, were compared with those produced by similar inocula of strain G in untreated birds (Table 1). Blood films

Table 1. *A comparison of the numbers of gametocytes produced by the sulphadiazine-treated strains of* Plasmodium gallinaceum, SD.A *and* SD.B, *and the untreated strain G*

| Day and time | Strain, no. of passage | Control | | SD 0·1 mg. | | SD 0·2 mg. | | SD 0·3 mg. | | SD 0·4 mg. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Gam. | P | Gam. | P | Gam. | P | Gam. | P | Gam. | P |
| 4th, 9.30–10.30 a.m. | G 17 | 14 | 349 | — | — | — | — | — | — | — | — |
| | SD.A 15 | 96 | 296 | 107 | 252 | 50 | 166 | 51 | 83 | — | — |
| 4th, 9.30–10.30 a.m. | G 18 | 10 | 288 | — | — | — | — | — | — | — | — |
| | SD.A 18 | 62 | 186 | 88 | 252 | 113 | 175 | 80 | 114 | 52 | 38 |
| 4th, 9.30–10.30 a.m. | G 53 | 23 | 406 | — | — | — | — | — | — | — | — |
| | SD.B 9 | 133 | 322 | — | — | 229 | 325 | 57 | 185 | 111 | 75 |
| 4th, 4–5 p.m. | G 53 | 17 | 387 | — | — | — | — | — | — | — | — |
| | SD.B 9 | 155 | 447 | — | — | 269 | 339 | 72 | 303 | 142 | 144 |
| 5th, 9.30–10.30 a.m. | G 53 | 15 | 215 | — | — | — | — | — | — | — | — |
| | SD.B 9 | 100 | 239 | — | — | 126 | 252 | 110 | 314 | 156 | 227 |
| 4th, 9.30–10.30 a.m. | G 55 | 13 | 332 | — | — | — | — | — | — | — | — |
| | SD.B 10 | 89 | 234 | 179 | 298 | 150 | 236 | 225 | 341 | 182 | 210 |
| 5th, 9.30–10.30 a.m. | G 55 | 13 | 301 | — | — | — | — | — | — | — | — |
| | SD.B 10 | 113 | 291 | 208 | 360 | 156 | 287 | 250 | 405 | 169 | 379 |

The dose of drug is given in mg./20 g. of body weight of bird. SD. = sulphadiazine. Gam. = the mean number of gametocytes per 10,000 erythrocytes in groups of up to six birds. P = the mean number of parasitized erythrocytes per 500 in groups of up to six birds.

were made twice daily, morning and evening, throughout the experiment, in order to follow the development of the gametocytes, but the counts were based on those made between 9·30 and 10·30 a.m. on the 4th day following inoculation. Although the intensity of infections produced by strain SD.A, particularly in birds treated with 0·2 and 0·3 mg. doses of the drug, was lower than that produced by strain G in untreated birds, the numbers of gametocytes were very much greater. Two birds in the SD.A series produced more than 200 gametocytes per 10,000 erythrocytes, whereas the highest gametocyte count in the birds of the strain G series was 29 per 10,000 erythrocytes. Not only were the numbers of gametocytes produced by strain SD.A high in birds treated with sulphadiazine, but they were also high

* Throughout this account, unless stated otherwise, dosage is given in mg. per 20 gm. of body weight of bird.

in untreated birds inoculated with this strain. The gametocytes increased in numbers gradually over the 4-day period.

After strain SD.A had been passed through a further series of birds treated with 0·3 mg. of sulphadiazine, making a total of eighteen passages through treated birds, the gametocyte-rate in the two strains was again compared. Again the gametocyte counts produced by strain SD.A were much higher, both in untreated birds and in those receiving 0·1, 0·2, 0·3 or 0·4 mg. doses of sulphadiazine, than in untreated birds infected with strain G (Table 1). Even in birds treated with 0·4 mg. of sulphadiazine, a dose which produced a low grade of parasitaemia, the numbers of gametocytes produced by strain SD.A were much greater than those produced by strain G in untreated birds though the infections produced by this latter strain were much heavier.

For comparison with strain SD.A, a second strain, SD.B, was isolated from the untreated strain G and passaged through birds treated with 0·1 mg. of sulphadiazine for 8 days, 0·2 mg. for 4 days, and 0·3 mg. for 24 days, the total number of serial passages during the entire period of treatment being 9. The numbers of gametocytes produced by this strain in untreated birds and in birds treated with 0·2, 0·3 and 0·4 mg. of sulphadiazine were compared with those produced by strain G in normal birds (Table 1). It was thought that the maximum numbers of gametocytes produced by the drug-treated strains and strain G might not synchronize; films were therefore made between 9.30 and 10.30 a.m. and 4 and 5 p.m. on the 4th day and between 9.30 and 10.30 a.m. on the 5th day following inoculation. In this experiment the number of birds was small, being only one for each dose of drug, and two for each set of untreated birds. In strain G the maximum count both of parasitized cells and gametocytes occurred on the 4th day, and, as in the previous experiments, the numbers of gametocytes produced by the sulphadiazine-treated strain both in untreated birds and in birds receiving 0·2, 0·3 or 0·4 mg. doses of the drug, greatly exceeded those produced by strain G in untreated birds.

After one further passage through a bird treated with 0·3 mg. of sulphadiazine (10th serial passage), the numbers of gametocytes produced by this strain and strain G were compared once more in groups of five to six chicks, in films made between 9.30 and 10.30 a.m. on the 4th day and 9.30 and 10.30 a.m. on the 5th day. As in former experiments, the numbers of gametocytes produced by strain G in untreated birds were few, the maximum being 29 per 10,000 corpuscles, whilst in three birds no gametocytes could be found though parasitaemia was heavy; the numbers produced by strain SD.B were much greater, several birds producing 250–300 gametocytes per 10,000 erythrocytes (Table 1). In only one bird treated with 0·3 mg. and in one treated with 0·4 mg. doses of sulphadiazine, did infections of more than twenty parasitized erythrocytes per 500 develop, but in both these birds the intensity of infection was high and gametocytes were numerous. This variability of infection in the groups of birds receiving the higher doses of drug suggested that resistance was not yet well developed in the strain.

A substrain of strain G was treated with proguanil for comparison with the two sulphadiazine-treated strains. The initial dose chosen (0·025 mg.) was one that had

little effect upon infections maintained by large inocula. After serial passage for 32 days through birds treated with this dose, it was raised to 0·05 mg. After serial passage for 28 days through birds receiving this amount of drug, making a total of sixteen passages over a period of 60 days, the numbers of gametocytes produced by this strain in untreated birds and in birds receiving 0·05 mg. doses of the drug were compared with the numbers produced by strain G in untreated birds (Table 2).

Table 2. *A comparison of the numbers of gametocytes produced by a proguanil-treated strain of* Plasmodium gallinaceum, *a strain treated with* 2:4-*diamino*-6:7-*diisopropylpteridine* (O/129 B) *the untreated strain G and the parent strain*

| Day and time | Strain, no. of passage | Control | | Proguanil 0·05 mg. | | Proguanil 0·1 mg. | | Proguanil 0·2 mg. | | O/129 0·1 mg. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Gam. | P | Gam. | P | Gam. | P | Gam. | P | Gam. | P |
| 4th, 9.30–10.30 a.m. | G 47 | 9 | 369 | — | — | — | — | — | — | — | — |
| | Prog. 16 | 102 | 381 | 108 | 384 | — | — | — | — | — | — |
| 4th, 9.30–10.30 a.m. | G 71 | 25 | 431 | — | — | — | — | — | — | — | — |
| | Prog. 34 | 57 | 302 | — | — | 113 | 322 | 103 | 280 | — | — |
| 4th, 9.30–10.30 a.m. | Parent 587 | 21 | 347 | — | — | — | — | — | — | — | — |
| | O/129 B 13 | 139 | 272 | — | — | — | — | — | — | 187 | 188 |

The dose of drug is given in mg./20 g. of body weight of bird. Prog. = proguanil-treated strain. O/129 B = 2:4-diamino-6:7-di*iso*propylpteridine-treated strain. Gam. = the mean number of gametocytes per 10,000 erythrocytes in groups of up to six birds. P = the mean number of parasitized erythrocytes per 500 in groups of up to six birds.

The numbers of gametocytes produced by the proguanil-treated strain were much greater than those produced by strain G, though not so great as in some of the birds inoculated with the sulphadiazine-treated strains. The proguanil-treated strain was passaged for a further period of 59 days through birds receiving 0·2 mg. doses making a total of thirty-four passages over a period of 154 days. The numbers of gametocytes produced by this strain in both untreated birds and birds receiving 0·1 or 0·2 mg. doses of proguanil once more exceeded the numbers produced by strain G in untreated birds, though, as in the previous experiment, the number produced in any one bird was not so great as the numbers produced in several birds inoculated with strains SD.A or SD.B.

In connexion with work upon the relationship of pteridine compounds to other antimalarials which are folic acid antagonists, a substrain, O/129 B, from the parent strain was being made resistant to 2:4-diamino-6:7-di*iso*propylpteridine by treatment with gradually increasing doses of that drug. It was passaged intravenously 13 times over a period of 71 days, through chicks receiving doses beginning with 0·05 mg. in the first passage, rising to 0·2 mg. twice daily. At the end of this period the numbers of gametocytes produced on the 4th day following inoculation were assessed, both in untreated birds and in birds receiving 0·1 mg. doses of the drug, and compared with the numbers produced by the parent strain in untreated birds. The numbers produced by strain O/129 B, both in untreated birds and in those receiving the drug, were much greater than those produced by the parent strain, the highest number in any one bird infected with strain O/129 B being 334 and the lowest 46, whereas the highest number produced by the parent

strain was 35 and the lowest 8. Although the numbers of gametocytes produced by strain O/129 were much greater than the numbers produced by the parent strain, parasitaemia was lower.

In all the experiments, films made immediately after the birds had received their inocula of 50 million parasites were examined, and a rough survey made of the numbers of gametocytes present in each bird. In no bird were gametocytes found in greater number than 1/25 fields. The large numbers of gametocytes found in birds inoculated with the drug-treated strains of parasite, therefore, were produced during the 5-day period of the experiment. The fact that the numbers of gametocytes present in the untreated birds inoculated with the drug-treated strains as well as in birds receiving drug were high, indicated that the increase in number of gametocytes was not due to the immediate and direct action of the drug. This deduction was confirmed by the following experiments.

Four young chicks were inoculated each with 0·1 ml. of heparinized blood from a chick heavily infected with the untreated parent strain. Two days later, when the infections were well-developed, two of the birds were treated with 1·25 mg. doses of sulphadiazine. This dose had a slow, but marked effect upon the asexual parasites, but the numbers of gametocytes present over the 4-day period of treatment did not differ significantly from those produced by the control birds. A similar experiment, using 0·05 mg. doses of proguanil, gave similar results.

No morphological difference could be detected, in films stained with Giemsa's stain, between gametocytes produced by the drug-treated strains, whether in birds treated with the drug or in untreated birds.

Exflagellation occurred as rapidly and as frequently in birds treated with the drugs as in untreated birds.

## DISCUSSION

In all the experiments in which the rate of gametocyte production in the drug-treated and untreated strains was compared, two points stand out clearly: the high proportion of gametocytes produced by the drug-treated strains, and the small proportion produced by the untreated strain G and the parent strain. The parent strain, of which strain G was a substrain, has been maintained since 1945 in a constant state of patent infection, solely by blood-inoculation. During this period it had become increasingly difficult to transmit it through mosquitoes.

A decrease in the numbers of gametocytes produced by strains of malaria parasites maintained by blood-inoculation, or even the loss of ability to produce them, has been noted by several workers. Thus Barzilai-Vivaldi & Kauders (1924), working upon a blood-inoculated strain of *P. vivax*, were unable to find any gametocytes in patients inoculated with the strain or to transmit it through *Anopheles maculipennis*; and similar observations were made by Cuboni (1926) and other workers. Later, Huff & Gambrell (1934) reported that a strain of *Plasmodium cathemerium* maintained in canaries for 7 months by blood-inoculation, suddenly gave rise to a gametocyteless substrain, whilst a second, poor in gametocytes but not devoid of them, was also derived from the same parent strain, which itself continued to produce gametocytes in large numbers. The gametocyteless strains still failed to produce gametocytes 12 years later (Huff & Coulston, 1946). More

recently, Lewert (1950) observed that when strains are maintained in such a way that the erythrocytic infections are so heavy that they cause the death of the host at an early stage of the infection, before exo-erythrocytic parasites become numerous, gametocytes become so few that mosquito infection is difficult or impossible. Gambrell (1937) had previously suggested that a long residence in one type of host, with constant asexual reproduction, tends to enhance the asexual characteristics and to diminish the sexual characteristics.

The method by which the parent strain and strain G had been maintained might, therefore, account for the small number of gametocytes produced in birds infected with them; but in the drug-treated strains large numbers of gametocytes were formed though the strains had been maintained by precisely the same method of passage.

Increases in gametocyte production were observed only in strains of *P. gallinaceum* treated with sulphadiazine, proguanil or 2:4-diamino-6:7-di*iso*propylpteridine. Although a substrain of the parent strain was made resistant to low doses of pamaquin (Bishop & McConnachie, 1952*b*) no increase in gametocyte production was observed. Strains were also treated with mepacrine (Bishop & Birkett, 1948) and chloroquine (Bishop & McConnachie, 1952*a*) for long periods, but failed to become resistant to them or to exhibit any significant change in the numbers of gametocytes produced. A strain of *P. gallinaceum* was made highly resistant to metachloridine (3-metanilamido-5-chloropyrimidine) (Bishop & McConnachie, 1953), but again no significant change in the proportions of gametocytes produced was observed throughout the period of treatment.

The three drugs which have produced strains of *P. gallinaceum* with high gametocyte counts are antagonists of the *p*-AB/folic acid system. Thus the antimalarial action of sulphadiazine is antagonized by *p*-aminobenzoic acid (*p*-AB) (Maier & Riley, 1942; Marshall, Litchfield & White, 1942; Bishop & McConnachie, 1948), and by pteroylglutamic acid (PGA) (Greenberg, 1949*a*); that of proguanil is partially antagonized by PGA (Greenberg, 1949*a*); but not by *p*-AB in *P. gallinaceum* (Bishop & McConnachie, 1948; Greenberg, 1949*b*), and by *p*-AB but not by PGA in *P. berghei* (Thurston, 1950), whilst that of 2:4-diamino-6:7-di*iso*propylpteridine in *P. gallinaceum* is antagonized by PGA but not by *p*-AB (Bishop, unpublished results).

From observations upon *P. gallinaceum* (Brackett, Waletzky & Baker, 1945), *P. falciparum* (Black, 1946), *P. vivax* (Mackerras & Ercole, 1948), *P. berghei* (Thurston, 1951, 1953) and *P. cynomolgi* (McFadzean, 1951), it can be concluded that proguanil, sulphadiazine and 2:4-diamino-6:7-di*iso*propylpteridine all act upon the early schizonts and prevent the normal processes of their division into merozoites. The drugs act much more slowly upon gametocytes than upon schizonts; indeed Mackerras & Ercole (1948) found that no morphological changes which could be attributed to the action of proguanil could be observed in gametocytes of *P. vivax* from patients treated with that drug, and they concluded that the final disappearance of these forms from the blood stream was due to natural causes and not to the action of the drug.

Evidence is accumulating which suggests that folic acid may play some part in the synthesis of nucleic acids. Thus Schöpfer (1948) found that, in *Eremothecium*

*ashbyii*, sulphonamide-inhibition was completely reversed by ribonucleic and desoxyribonucleic acids. Prussoff, Tepley & King (1948) describe a marked decrease in desoxyribonucleic acid in *Lactobacillus casei* when growth was restricted by a deficiency of pteroylglutamic acid in the medium. However, in *Leuconostoc citrovorum* cells, harvested from medium partially deficient in citrovorum factor but otherwise favourable to growth, Chang, Silverman & Keresztesy (1951) found a higher concentration of desoxyribonucleic acid and ribonucleic acid than in normal cells.

Multiple injections of Aminopterin or A-methopterin, which are both folic acid antagonists, into normal mice, profoundly decreased the rate of synthesis of nucleic acid. These drugs were, also, found to inhibit the incorporation of $^{14}C$ from formate into nucleic acids and nucleic acid purines (Skipper, Mitchell & Bennett, 1950).

The Feulgen technique for the detection of desoxyribonucleic acid (DNA) has been applied to several species of Plasmodia. Jírovec & Černý (1932) found that in *Plasmodium praecox* the multinucleate schizonts gave a strong reaction, whereas none was obtained from gametocytes or young schizonts. Chen (1944) obtained a strong reaction in trophozoites and schizonts of *P. elongatum*; most of the gametocytes remained colourless, though a few stained faintly. In *P. vivax* and *P. knowlesi* merozoites and ring forms stained most intensely, trophozoites and the earlier stages of schizogony less intensely, whilst gametocytes stained very faintly (Deane, 1945). Lewert (1952*a*) found an increase in the intensity of the reaction, in *P. gallinaceum*, as schizogony progressed, whereas the gametocytes gave no reaction. The present writer has applied the technique described by Deane to blood films heavily infected with the parent strain of *P. gallinaceum*, and has also found that the intensity of the staining reaction increases with the development of the schizont, and that the nuclei of the mature schizont and of the merozoites stain deeply, whereas the nuclei of the gametocytes do not stain at all.

Estimations of the nucleic acids present in *P. berghei*, by biochemical methods, were made by Whitfeld (1952). The nucleic acids present were similar to those of other organisms, the RNA content being about twice the DNA content.

It has been shown, in vertebrates, that the amount of DNA present in the somatic cells of an individual and of all the individuals of a species, is constant, the haploid sperm nuclei containing half the amount present in the somatic cells (Boivin, Vendrely & Vendrely, 1948; Vendrely & Vendrely, 1949, 1950). According to Mirsky & Ris (1949), differences based on staining, described by some workers, in the quantity of DNA in different cells, result from alterations in the percentage composition due to changes in the quantity of the residual protein, the amount of DNA being constant. If such observations, based upon the nuclei of higher animals, hold good for the nuclei of the malaria parasite, then the apparent lack of DNA in the gametocytes may be due to the dispersal of the small amount of DNA, presumably equal to that in a single merozoite, through the much larger nuclear mass of the gametocytes.

The possibility that folic acid is concerned in the synthesis of nucleic acids suggests an explanation of the schizonticidal action of folic acid antagonists such

as proguanil, sulphadiazine and 2:4-diamino-6:7-di*iso*propylpteridine and their lack of action against gametocytes. The ultraviolet light absorption measurements of large, proguanil-treated parasites were found by Lewert (1952*b*) to show a lower level of nucleotides and a decrease in protein as compared with normal parasites of the same size. The drug was found to interfere with the accumulation of ribonucleic acid (RNA) in the cytoplasm of the parasite.

Although the action of proguanil, sulphadiazine and 2:4-diamino-6:7-di*iso*propylpteridine appears to be directed, in the first instance, against nuclear division, exflagellation has been observed in blood taken from patients undergoing treatment with proguanil, though zygotes, if formed, were unable to complete their development in the stomach of mosquitoes fed upon such patients (Fairley, 1946). The gametocyte nucleus divides prior to exflagellation, to provide the nuclei of the male gametocytes, but, though Lüdicke & Piekarski (1952) state that these nuclei are Feulgen-positive, nothing is known, as yet, of the development of DNA or RNA prior to the formation of the gametes. If the DNA present in the resting nuclei of malaria parasites is constant, as in higher animals, then sufficient must be synthesized by the male gametocyte in the presence of the drug, to provide the male gametes with the required amount.

Great variations occur in the numbers of gametocytes produced by the same strain of malaria in different individuals. Thus, Bishop (1943) found that blood rich in gametocytes, from canaries infected with *P. relictum*, did not necessarily produce a high gametocyte count in the recipient canaries, and samples of blood from one source, often produced infections which differed considerably in the numbers of gametocytes formed. The age of the recipients did not appear to be responsible for the variation. More recently the present writer has had occasion to observe wide variations in the numbers of gametocytes produced in young chicks by pooled sporozoites from a mosquito-transmitted strain of *P. gallinaceum*.

However, although the numbers of gametocytes produced by the drug-treated strains varied from bird to bird, they were invariably greater than those produced by the untreated strains. It can, therefore, be assumed that the greater numbers of gametocytes produced by the drug-treated strains were due to the action of the drugs. The effect of proguanil, sulphadiazine and 2:4-diamino-6:7-di*iso*propylpteridine upon gametocyte production appears to be a gradual one, and is particularly marked at times when the division rate is slightly depressed. These observations might suggest that the increase in gametocyte production may result from the inability of some parasites to synthesize nuclear material at the normal rate required for the rapid nuclear division required by schizonts. It is too early, however, to make such a deduction as so little is known of the conditions which govern gametocyte production.

## SUMMARY

In strains of *Plasmodium gallinaceum* passaged by blood-inoculation, through young birds treated with small, but effective doses of proguanil, sulphadiazine or 2:4-diamino-6:7-di*iso*propylpteridine, the numbers of gametocytes produced were much greater than in the parent strain passaged by the same method.

The effect of the drugs upon gametocyte production appears to be a long term one, since no change in gametocyte numbers was observed in birds treated with proguanil or sulphadiazine for only a few days.

The relationship of these drugs to nuclear division and the synthesis of nucleic acids is discussed in the light of the observed increase in gametocyte-production in drug-treated strains of *P. gallinaceum*.

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# THE ACTION OF AMOEBICIDAL DRUGS ON *ENTAMOEBA INVADENS* RODHAIN, 1934 *IN VITRO*

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## INTRODUCTION

Little is known of the effect of drugs on *Entamoeba invadens* Rodhain, 1934, the intestinal parasite of reptiles first described by Ratcliffe & Geiman (1933, 1934) and Rodhain (1934). Rodhain & Van Hoof (1935) found that by feeding cultures of *E. invadens* containing cysts to reptiles, they were able to produce all the symptoms of amoebiasis, while the addition of emetine hydrochloride to the cultures, in concentrations of 1/5000, prevented the development of amoebae, and of intestinal and liver lesions in reptiles inoculated with such cultures. No attempt, however, was made by these workers to determine the sensitivity of *E. invadens* to emetine. Lamy & Chevrier (1948) claim to have demonstrated a specific action of a sulphonamide against *E. invadens*, and not against *E. histolytica*, but this observation requires confirmation, as it is not known if the bacterial flora accompanying the two species of *Entamoeba* in their tests were the same.

The marked resemblances in morphology, life cycle and pathogenicity between *E. invadens* and *E. histolytica* (Geiman & Ratcliffe, 1936; Ratcliffe & Geiman, 1938) suggested that an investigation of the response of the former species to amoebicidal drugs would be of interest. If the sensitivity of the two organisms *in vitro* to emetine and other amoebicidal drugs were found to be similar, *E. invadens* would be particularly suited for the *in vitro* screening of potential amoebicides, since, unlike *E. histolytica*, it is non-pathogenic to man, and cultures of the organism can be manipulated at room temperature. A method was therefore required by which the sensitivity of *E. invadens* to the action of amoebicidal drugs *in vitro* could be determined under conditions which would permit of a comparison with the sensitivity of *E. histolytica* to such drugs.

Since Dobell & Laidlaw (1926) studied the action of emetine on *E. histolytica* growing in culture, numerous *in vitro* tests on the action of emetine and other compounds on this organism have been described. Having regard to emetine alone, the outstanding feature of these tests has been the diversity of results obtained by different workers; for instance, the minimum effective dose of emetine is recorded as varying from 1/10,000 (Anderson & Chuan, 1944; Conan, 1948; Ishii, 1948) to 1/5,000,000 (Laidlaw, Dobell & Bishop, 1928; St John, 1933; Dobell, 1947; Fulton, Joyner, King, Osbond & Wright, 1950). It is now known that the activity of amoebicidal drugs *in vitro* may be influenced by several factors.

Laidlaw *et al.* (1928) demonstrated that emetine, an alkaloid, was absorbed by the solid part of the medium when tested in the diphasic media first used for cultivating *E. histolytica*; the amount of drug remaining in the liquid part of the medium and acting upon the amoebae was thus unknown, and with such a variable

factor, the test could have little value for comparisons of relative activities of drugs. These workers successfully used a wholly liquid medium for the cultivation of *E. histolytica*, and advocated its use for all *in vitro* drug tests. The persistence of many workers in using a diphasic medium renders their results of little value; the high minimum effective doses reported for emetine by Anderson & Chuan (1944) and Ishii (1948) were obtained using diphasic media, and have no comparative value with the results obtained in wholly liquid media.

The activity of emetine is also influenced by pH, the drug being considerably more active in alkaline than in acid media (Laidlaw *et al.* 1928; St John, 1933). Tests on the action of amoebicidal drugs should therefore be carried out under defined conditions of pH, and the incorporation of a buffer, as proposed by Laidlaw and his colleagues, tends to stabilize the reaction of the test medium.

The time for which the amoebae are exposed to the action of a drug is also of importance in determining its activity. Thus solutions of emetine of 1 % or more are needed to kill *E. histolytica* instantaneously, whereas much weaker solutions of the same drug will kill all the amoebae, if allowed to act for a sufficient time (Dobell & Laidlaw, 1926). Many drugs, of which emetine is probably one (Stewart, 1949), appear to act only on a growing population, and should therefore be given time in which to exert their full effects.

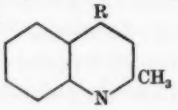
The presence of bacteria in cultures of *E. histolytica* is an additional complicating factor in *in vitro* drug tests. A drug which inhibits the growth of the bacteria may indirectly inhibit the growth of the amoebae, and it is necessary to distinguish between antibacterial and amoebicidal activity in tests designed to determine the specific amoebicidal action of drugs. Dobell & Laidlaw (1926), aware of this, concluded that emetine had a specific action on *E. histolytica*, because in amoebicidal concentrations it did not appear to inhibit the bacteria, and because *E. coli*, which is equally dependent on bacteria in culture, was not inhibited by emetine in concentrations lethal to *E. histolytica*. In cultures in which amoebae are growing with a mixed bacterial flora, however, it is impossible to detect whether a drug acts selectively against one species of bacterium, without affecting the overall bacterial growth. Selective inhibition of one species of bacterium might affect the growth of the amoebae.

Several methods have now been devised for distinguishing between antibacterial and amoebicidal activity in *in vitro* drug tests on *E. histolytica*. The development of cultures of *E. histolytica* growing in the presence of *Trypanosoma cruzi* alone (Phillips, 1950; Phillips & Rees, 1950) has provided a strain particularly suitable for use in drug tests, as *T. cruzi* is, in general, more resistant to drugs than *E. histolytica*, and any trypanocidal action can be determined by manometric measurements of the respiration of the trypanosomes in the presence of the drugs (Nakamura & Anderson, 1951). The tests described by Dobell (1947) and Hansen (1949) were both designed for use with monobacterial strains of *E. histolytica*, and thus eliminate the possibility of selective inhibition of one species of bacterium. In Dobell's test, amoebae from a strain growing with *Bacterium coli* alone were inoculated into buffered liquid horse serum-Ringer containing starch, methylene blue and the concentration of emetine under test. Before inoculating the culture medium with

amoebae, the tubes were seeded with a few drops of a broth culture of *Bact. coli*; after 24 hr. incubation, the bacterial growth was sufficient to decolorize the methylene blue, and the failure of any of the tubes to lose their blue coloration was taken as an indication that the bacterial growth had been inhibited by the drug, and that such concentrations of drug were not suitable for testing against the amoebae. Hansen (1949) proposed the use of petrolatum-sealed cultures in tests to determine the action of drugs on a strain of *E. histolytica* growing with a single species of bacterium (organism *t*). This test is superior to Dobell's test in that an amoebicidal end-point can be obtained for drugs which inhibit the growth of the bacteria; the anaerobic conditions in the sealed cultures permit of the multiplication of the amoebae, unless amoebic growth is specifically inhibited by the drug.

The conditions essential for amoebicidal drug testing would thus seem to be: a medium which does not remove by absorption any of the drugs under test; a known pH, maintained in the region of neutrality throughout the test; an incubation period long enough to permit the drugs to exert their maximum activity; and a technique for detecting any antibacterial action of the drugs under test. Even when all these conditions are fulfilled, it is legitimate to compare the results of any two tests only when they have been carried out under as nearly identical conditions as possible; different media, and differing species of accompanying bacteria may affect the growth of the amoebae, and thus the final end-point.

The action of certain drugs upon *E. invadens in vitro* was therefore tested by a method which fulfilled the above conditions, and which resembled as closely as possible the conditions in tests on *E. histolytica*, in order that the response of the two organisms to these drugs might be compared. The method used was based on that devised for determining the action of emetine on *E. histolytica* by Dobell (1947), and which was used by Fulton and his collaborators to determine the effect of a series of compounds on *E. histolytica* (Fulton *et al.* 1950). These workers discovered several compounds derived from 4-chloroquinaldine and 2-anilino-4-chloroquinoline, with an activity only slightly less than that of emetine, when tested under the same conditions. In addition to emetine, the drugs chosen for the present tests on *E. invadens* were derivatives of 4-chloroquinaldine, compounds A 352, A 356 and A 404.




A 352 – R = .NH.$(CH_2)_2$.NH.$C_9H_{19}$ *n*,

A 356 – R = .NH.$(CH_2)_2$.NH.$C_9H_{17}$ *n*,

A 404 – R = .NH.$C_9H_{19}$ *n*.

## THE SENSITIVITY OF *E. INVADENS IN VITRO* TO EMETINE, A 352, A 356 AND A 404

### *Experimental methods*

A monobacterial strain of *E. invadens* was not available at the time when the sensitivity of the organism to emetine and the quinaldines was first determined, and the tests were made using a strain (strain F) which was growing in the presence of a strain of *Bact. coli* (A′) and an unidentified bacterium, designated organism *x*. Later, the sensitivity to emetine of a strain of *E. invadens* (strain GV), growing with

*Bact. coli* A′ alone, was determined. These strains were produced, by a method to be described elsewhere, from a strain of *E. invadens* which was isolated by Prof. J. Rodhain, and given to the Molteno Institute by Dr L. Lamy. The strain of *Bact. coli* present in these strains was identical with that in the 'pure mixed' strain of *E. histolytica* obtained by Dobell (1947, 1952). Stock cultures of both strains were maintained in liquid horse serum-Ringer (1:7) containing starch (*hs*+*S*), in which medium mass encystation was never observed, although cysts were occasionally found in comparatively small numbers.

The culture medium used in preliminary experiments on the action of emetine on strain F was inactivated horse serum-Ringer (1:7), buffered with 0·2 % $Na_2HPO_4$. In later experiments, the 0·2 % $Na_2HPO_4$ was replaced by a phosphate buffer of the following composition: 87 ml. M/15-$Na_2HPO_4$ in Ringer's solution (0·946 g./100 ml.) added to 13 ml. M/15-$KH_2PO_4$ in Ringer's solution (0·906 g./100 ml.). This medium (*hsph*) was used in all the tests on the action of emetine, A 352, A 356 and A 404 on strains F and GV, the pH of the fresh medium being 7·4–7·5. One ml. of a 0·1 % aqueous solution of methylene blue was added to every 100 ml. of Ringer phosphate, as recommended by Fulton *et al.* (1950). The Ringer phosphate was sterilized by autoclaving at 15 lb. pressure (120° C.) for 20 min., and was mixed in the proportion of 7:1 with sterile inactivated horse serum.

Stock solutions of emetine hydrochloride and the quinaldines were made up in sterile Ringer phosphate containing methylene blue, and steamed for ½ hr., the solutions being tested for sterility by incubation at 37° C. for 24 hr., and by inoculation of small amounts into glucose broth, followed by incubation of the broth for 24 hr. at 37° C. The dilutions of drugs for testing were prepared by mixing the stock drug solutions with the *hsph* medium in the required proportions, all the dilutions being carried out under sterile conditions. The medium was tubed in 5 ml. amounts and tested for sterility by incubation, as before. One loopful of sterile rice starch was then added to each tube of medium, and after a further 24 hr. incubation, 5 drops of a 24 hr. culture of *Bact. coli* A′ in 2 % glucose broth were added to each tube. After 24 hr. incubation at 37° C., when there was good growth of bacteria, with decoloration of methylene blue, the tubes were ready for inoculation with amoebae. Any tubes in which the bacterial growth was inhibited by the concentration of drug present were detected by the persistence of the blue coloration, and such concentrations of drug were not tested against the amoebae.

The amoebae for inoculation were obtained by pooling two to three stock cultures showing good growth in *hs* medium. After suspending the amoebae evenly through the medium, approximately equal volumes were inoculated into each of two tubes of each concentration of drug to be tested, and into two control tubes, containing no drug. When the quinaldines were tested, a series of concentrations of emetine was also included, as a standard. The cultures were then incubated at 24° C. The growth of amoebae was observed microscopically on the 5th and 10th days after inoculation, and subcultures into *hs*+*S* medium made from all 'negative' cultures—i.e. cultures in which no amoebae, or only apparently dead amobae, were found. These subcultures were examined at intervals of 5 days for periods up to

4 weeks, in order to determine if there were any living amoebae in the original inocula from the test cultures.

The pH of the medium was measured at the time of inoculation of the amoebae (i.e. after *Bact. coli* A′ had grown in the medium for 24 hr.), and at the end of the test. It was found most convenient to prepare three tubes of medium for each concentration of drug, and to determine the pH of one of each set of tubes at the beginning of the experiment, before inoculating the remaining two with amoebae. A Cambridge pH meter and a glass electrode were used for the determinations.

*Results*

When the action of emetine on strain F of *E. invadens* was tested in medium buffered with 0·2 % $Na_2HPO_4$, it was found that the pH of the medium at the time of inoculation of the amoebae was below 7·0 in all the cultures, in some as low as 6·3. After 5 days' incubation the pH had risen slightly, but in the majority of cultures it was still below 7·0. Considerable variation in pH was observed in different tests —for instance, in one test, the pH at the beginning of the experiment ranged between 6·3 and 6·7, and in another, between 6·6 and 6·9; under these conditions, the minimum effective dose of emetine was not clearly defined. Emetine was found to be considerably less active in the more acid media, as shown by Laidlaw *et al.* (1928) and St John (1933).

It was considered that a more sharply defined end-point might be obtained by stabilizing the pH of the medium more precisely than is possible with $Na_2HPO_4$ alone. Assuming that it is the base of emetine which is the active agent (Laidlaw *et al.* 1928), it seemed that optimum conditions for testing its action would be obtained by maintaining the medium at a pH above neutrality for the whole of the time the amoebae were in contact with the drug. Such conditions were realized by substituting the phosphate buffer previously described for the 0·2 % $Na_2HPO_4$ used by Dobell. From Tables 1 and 2 it can be seen that at the time of inoculation of the amoebae, the pH of cultures so buffered was, in the majority of cultures, 7·0–7·1 and never fell below 6·7, while after 10 days' incubation, it was always above 7·0. Growth of amoebae in medium so buffered was as good as in that buffered with $Na_2HPO_4$ alone, or in unbuffered *hs*.

The results of the tests made to determine the effect of emetine and the quinaldines upon the dibacterial strain F are shown in Table 1, and of emetine upon the monobacterial strain GV, in Table 2; the sensitivity of the two strains to emetine was identical. Defining the minimum inhibitory dose as the lowest dose inhibiting the growth of the amoebae, so that no living organisms were found in the cultures when examined at the end of a 10-day period, and the minimum lethal dose as the lowest dose killing all amoebae, as determined by subcultures from apparently negative cultures at the end of a similar period, the results given in Table 3 were obtained. The concentrations of drugs tested for amoebicidal activity had no effect on bacterial growth, as determined by reduction of methylene blue and direct observation of the intensity of bacterial growth. As A404, 1/5000 and A352, 1/10,000 inhibited the bacteria so that methylene blue was not decolorized after

Table 1. *The sensitivity of the dibacterial strain F of* Entamoeba invadens *to emetine and the quinaldines A* 352, *A* 356 *and A* 404.

| Drug | Dose | pH at time of inoculation of amoebae | Mean pH at end of test | Result* determined by observation. Incubation period (days) 5 | Result* determined by observation. Incubation period (days) 10 | Result* determined by subculture. Incubation period (days) 5 | Result* determined by subculture. Incubation period (days) 10 |
|---|---|---|---|---|---|---|---|
| Exp. 1 | | | | | | | |
| Control | — | — | 7·5 | 2 | 2 | — | — |
| Emetine | 1/100,000 | 7·0 | 7·5 | 0 | 0 | 2 | 2 |
| Emetine | 1/500,000 | 7·0 | 7·5 | 2 | 2 | — | — |
| Emetine | 1/1,000,000 | 7·1 | 7·4 | 2 | — | — | — |
| A 352 | 1/50,000 | 7·1 | 7·4 | 0 | 0 | 0 | — |
| A 352 | 1/100,000 | 7·0 | 7·5 | 0 | 0 | 0 | 0 |
| A 404 | 1/10,000 | 7·0 | 7·4 | 0 | 0 | 0 | 0 |
| Exp. 2 | | | | | | | |
| Control | — | — | 7·3 | 2 | 2 | — | — |
| Emetine | 1/50,000 | 6·9 | 7·3 | 0 | 0 | 1 | 0 |
| Emetine | 1/100,000 | 7·0 | 7·4 | 0 | 0 | 1 | 1 |
| Emetine | 1/500,000 | 6·8 | 7·3 | 2 | 2 | — | — |
| A 352 | 1/100,000 | 6·9 | 7·3 | 0 | 0 | 0 | 0 |
| A 352 | 1/500,000 | 6·9 | 7·2 | 2 | 2 | — | — |
| A 356 | 1/100,000 | 6·9 | 7·2 | 0 | 0 | 1 | 1 |
| A 404 | 1/10,000 | 6·7† | 7·1 | 1 | 0 | 2 | 0 |
| A 404 | 1/50,000 | 6·9 | 7·2 | 1 | 1 | 2 | 2 |
| Exp. 3 | | | | | | | |
| Control | — | — | 7·4 | 2 | 2 | — | — |
| Emetine | 1/100,000 | 7·0 | 7·5 | 1 | 0 | 2 | 0 |
| Emetine | 1/500,000 | 7·1 | 7·5 | 1 | 0 | 2 | 2 |
| A 356 | 1/50,000 | 7·0 | 7·5 | 0 | 0 | 1 | 0 |
| A 356 | 1/100,000 | — | 7·5 | 0 | 0 | 2 | 0 |
| A 356 | 1/200,000 | 7·1 | 7·4 | 2 | 2 | — | — |

* Expressed as the number of positive cultures, out of two cultures inoculated.
† Determined on a sample of less than 5 ml.

Table 2. *The sensitivity of the monobacterial strain GV of* Entamoeba invadens *to emetine*

| Drug | Dose | pH at time of inoculation of amoebae | Mean pH at end of test | Result* determined by observation. Incubation period (days) 5 | Result* determined by observation. Incubation period (days) 10 | Result* determined by subculture. Incubation period (days) 5 | Result* determined by subculture. Incubation period (days) 10 |
|---|---|---|---|---|---|---|---|
| Control | — | 7·1 | 7·2 | 2 | 2 | — | — |
| Emetine | 1/50,000 | 7·1 | 7·3 | 0 | 0 | 1 | 0 |
| Emetine | 1/100,000 | 7·1 | 7·4 | 0 | 0 | 1 | 0 |
| Emetine | 1/500,000 | 7·1 | 7·4 | 2 | 2 | — | — |

* Expressed as the number of positive cultures, out of two cultures inoculated.

24 hr. incubation at 37° C., these concentrations of drugs were not tested for amoebicidal activity.

Table 3. *A comparison of the sensitivities of* Entamoeba invadens *and* Entamoeba histolytica *to emetine and the quinaldines A* 352, *A* 356 *and A* 404

| | Emetine | A 352 | A 356 | A 404 |
|---|---|---|---|---|
| | *E. histolytica* | | | |
| M.E.D. (Fulton *et al.* 1950) | 1/100,000 to 1/5,000,000 | 1/100,000 to 1/500,000 | 1/100,000 to 1/500,000 | 1/10,000 to 1/100,000 |
| | *E. invadens* | | | |
| M.L.D. | 1/50,000 to 1/100,000 | 1/100,000 | 1/100,000 | 1/10,000 |
| M.I.D. | 1/100,000 to 1/500,000 | 1/100,000 | 1/100,000 | 1/10,000 to 1/50,000 |

M.E.D. = minimum effective dose; M.L.D. = minimum lethal dose; M.I.D. = minimum inhibitory dose.

Incubation period for *E. histolytica*, 3 days; incubation period for *E. invadens*, 10 days.

It was found that emetine was active against *E. invadens* over a wide range of concentrations. Amoebae were never found by microscopic observation in concentrations of 1/50,000, while at 1/100,000 one motile amoeba was found on only one occasion after 5 days' exposure to the drug, such cultures usually being completely negative or containing a few abnormal amoebae; after 10 days' exposure to this dose of emetine, amoebae were never found. The effect of the drug at a concentration of 1/500,000 was variable; very occasionally no amoebae were found after 5 days, but in most tests growth occurred, though considerably reduced compared with that of the controls, and abnormal amoebae were present. Cultures which showed a reduced growth after 5 days' exposure to emetine 1/500,000 were sometimes negative after a further 5 days' incubation in the presence of the drug, and sometimes showed a growth as good as that in the controls, though abnormal amoebae could still be found. Even when no amoebae were observed microscopically, living amoebae occasionally survived, as positive subcultures were sometimes obtained from 'negative' test cultures containing emetine in concentrations of 1/50,000–1/500,000 (Tables 1 and 2). At a concentration of 1/1,000,000 normal amoebae were always present on the 5th day, though in one test, abnormal amoebae were also present, and growth was reduced compared with that in the controls. Cultures containing emetine in this concentration and incubated for 10 days, produced a growth of amoebae comparable to that in the control cultures.

In contrast to emetine, the end-points of compounds A 352, A 356 and A 404 were more sharply defined. Cultures exposed to a concentration of 1/100,000 of A 352 or A 356 were invariably negative, as determined by microscopic observation, after 5 or 10 days' incubation, while at 1/500,000 growth in the majority of cultures was comparable to that of the controls, though a few abnormal amoebae were occasionally found. Compound A 404, in concentrations of 1/10,000, inhibited growth so that in all but one test, no amoebae could be found after 5 or 10 days; in one test, a single motile amoeba was observed in one culture after 5 days'

incubation, but this culture was completely negative after a further 5 days' incubation. In cultures containing A 404 1/50,000, growth was markedly reduced after 5 or 10 days, and in one test no amoebae were observed, but at a concentration of 1/100,000 growth was as heavy as in the controls. As with emetine, doses of the quinaldines which inhibited growth were not invariably lethal (Table 1).

The appearance of abnormal amoebae in cultures exposed to the action of emetine or the quinaldines was characterized by an absence of starch grains and loss of motility, with vacuolation of the cytoplasm, which became somewhat granular. Disintegrating amoebae were also found in cultures affected by the drugs.

## THE EFFECT OF PROLONGED EXPOSURE TO EMETINE ON A STRAIN OF *ENTAMOEBA INVADENS*

A substrain of strain F (designated strain E) was maintained in medium containing increasing concentrations of emetine, in order to determine if *E. invadens* could acquire resistance to the drug. It was thought that resistance was most likely to arise if the amoebae were first maintained in a concentration of drug which had only a slight effect on their growth, since resistant mutants, if they occurred, would be most likely to arise in a growing population. Accordingly, strain E was maintained for seven subcultures, made at intervals of approximately 10 days, in *hsph*+*S* containing emetine hydrochloride in a concentration of 1/10,000,000, a dose considerably less than the minimum inhibitory dose in the drug tests. Heavy growth was obtained in all the cultures, although a few degenerate amoebae were occasionally found. The strain was then transferred for five subcultures to medium containing emetine 1/5,000,000, followed by five subcultures in the presence of emetine 1/1,000,000; the growth of the amoebae did not appear to be affected by emetine in either of these concentrations, although degenerate amoebae were again occasionally found. Strain E was next maintained in medium containing emetine in a concentration of 1/500,000; the numbers of amoebae in such cultures were considerably less than in the lower concentrations, and many abnormal amoebae were found in the sixth subculture, while the seventh subculture in medium containing emetine 1/500,000 was completely negative, and no amoebae were recovered on subinoculation into normal *hs*. Strain E was maintained for 9 months in the presence of emetine in concentrations increasing from 1/10,000,000 to 1/500,000, but the amoebae died out after several transfers through medium containing the latter concentration of drug. They had thus acquired no resistance to the minimum concentration of emetine which inhibited the growth of *E. invadens*.

## DISCUSSION

In comparing the activity of emetine and the quinaldines upon *E. invadens* with their activity upon *E. histolytica*, the tests on *E. invadens* were designed to conform as closely as possible to the conditions under which the tests upon *E. histolytica* were carried out by Dobell (1947) and Fulton *et al.* (1950). Certain differences, however, could not be avoided, the most serious being that strain F of *E. invadens* was growing with a mixed bacterial flora, namely *Bact. coli* A′ and organism *x*. Since, however, the sensitivity to emetine of the monobacterial strain GV was

identical with that of strain F, it was concluded that the presence of organism *x* did not affect the response of *E. invadens* to drugs. The other differences between the tests on *E. invadens* and those on *E. histolytica* were first, the temperature, which in the tests upon *E. histolytica* was 37° C., and in those on *E. invadens* was 24° C.; secondly, the incubation period (3–4 days for *E. histolytica*, 5–10 days for *E. invadens*); and thirdly, the method by which the pH of the medium was stabilized. *E. invadens*, which grows at a lower temperature than *E. histolytica*, appears to have a lower multiplication rate, and heavy growth in control cultures was obtained only after 10 days' incubation, though after 5 days, the controls always showed a good growth. In certain tests (Tables 1 and 2), it was found that drugs which failed to kill all the amoebae in 5 days, did so after 10 days. An incubation period of 10 days is therefore desirable for drug tests on this organism. It is unlikely that the use of $Na_2HPO_4$–$KH_2PO_4$ as buffer, as opposed to $Na_2HPO_4$ alone, influenced the result of the tests, since the object of using a buffer—to maintain a pH at which emetine could exert its maximum activity—was apparently achieved by both methods. Dobell and Fulton and his colleagues gave no details of the pH of their test media at the time of inoculation of the amoebae, or at the end of the test, so the variation of pH in these tests is not known. In the tests upon *E. invadens*, a more stable pH may have been necessary, since this organism appears to be less sensitive to emetine than is *E. histolytica*; a slightly acid medium, which would inactivate some of the drug present, could thus produce a sublethal concentration of emetine.

In spite of these discrepancies between the two tests, it seems legitimate to compare the results obtained for *E. invadens* with those of the tests upon *E. histolytica*, since the medium and, as far as is known, the pH were the same, while in the tests on the sensitivity of the monobacterial strain GV, the bacterial flora was identical with that in the strain of *E. histolytica* used by Dobell and by Fulton and his colleagues. The effects of emetine and the compounds A 352, A 356 and A 404 appear to be similar on the two species, though these strains of *E. invadens* are less sensitive to emetine than Dobell's 'pure mixed' strain of *E. histolytica* (Table 3). Dobell records an end-point of 1/5,000,000 and Fulton and his colleagues of 1/100,000–1/5,000,000 for emetine on *E. histolytica*. The lowest concentration which killed *E. invadens* in some of the tests was 1/100,000, and although this concentration, or even one of 1/50,000, was not always lethal, it invariably inhibited growth to such an extent that living amoebae were never observed microscopically in the cultures at the end of the tests. A concentration of 1/500,000 markedly inhibited the growth of the amoebae, and in one test growth was also reduced by a concentration of 1/1,000,000. It is possible that the end-point was determined more accurately in the present tests than in those of Fulton and his collaborators, since these workers state that subcultures were made from test cultures 'in cases of doubt'. In the present tests, subcultures were made from all 'negative' cultures, and it was found that cultures in which no amoebae were observed microscopically, frequently gave rise to positive subcultures. Fulton's minimum effective dose may thus sometimes have corresponded to the minimum inhibitory dose, as defined in the present work, and not to the minimum lethal dose. It is also possible that in

the present tests positive subcultures may have been due to the survival of cysts in the test cultures, although cysts were never observed, even in the control cultures. Observations on several strains of *E. invadens* have indicated that cysts are formed only after a period of fairly heavy growth of amoebae; thus any cysts present would have been introduced in the original inocula into the test cultures, as subcultures were made only from cultures in which no growth of amoebae had occurred. The possibility that this may have occurred cannot be excluded, as cysts have been found occasionally in stock cultures of both strains F and GV, growing in *hs+S*; the survival of cysts, however, would have affected the value of the minimum lethal dose only, and not that of the minimum inhibitory dose. The strain of *E. histolytica* used by Dobell and by Fulton and his collaborators was admirably suited for use in drug tests, as it was a non-encysting strain. The activity of A 352, A 356 and A 404 upon *E. invadens* was closer to their activity on *E. histolytica* than was that of emetine (Table 3); for all three, the minimum lethal dose for *E. invadens* was within the range found for *E. histolytica*.

The strains of *E. invadens* used in the present tests have been shown to be less sensitive to the action of emetine than is *E. histolytica*. Indeed, *E. invadens* survived doses of emetine which are toxic to *E. coli*, the intestinal amoeba whose *in vivo* resistance to emetine has long been known; Bishop (1929) obtained an end-point of 1/300,000–1/600,000 for emetine on *E. coli* growing in buffered serum-Ringer, with a pH range of 6·8–7·2. It is possible that the difference in response to emetine of *E. invadens* and *E. histolytica* may be due to a difference in metabolic rate, rather than to some fundamental difference in metabolism; it is known that high dilutions of emetine are active only against growing populations of *E. histolytica* (Stewart, 1949), and the slower growth rate of *E. invadens* may render the organism less susceptible to the action of emetine. It is suggested that the difference in response to emetine of *E. histolytica* and *E. coli*, which occur in the same environment, may be more fundamental than that of *E. histolytica* and *E. invadens*, which grow at different temperatures. Whether the quinaldines act in a similar way to emetine is not known, but the sensitivity of *E. invadens* to these compounds is very similar to that of *E. histolytica*, and it would be of interest to determine the action of drugs of a more varied chemical composition on *E. invadens*. The present results indicate that it is unlikely that *E. invadens* will replace *E. histolytica* for *in vitro* testing of drugs; nevertheless, *E. invadens* may be of value in screening new compounds for amoebicidal activity.

Several reports have been made of a change in sensitivity to emetine in strains of *E. histolytica* which were maintained in media containing increasing concentrations of the drug, but as Jones (1952) pointed out, there is little justification for the conclusions of Halawani (1930) and Bonnin & Aretas (1938, 1939) that *E. histolytica* may acquire resistance to emetine; in their experiments, for instance, the strains of *E. histolytica* were maintained in diphasic media, whose pH was not controlled, and under such conditions, tests on the effect of emetine have little significance. Jones was unable to induce resistance in a strain of *E. histolytica* which was maintained for 5 months in liquid media, of known and constant pH, containing sublethal concentrations of emetine. Similarly, in the present work,

a strain of *E. invadens* did not become resistant to emetine after it had been maintained for 9 months in liquid media, also of constant pH, containing sublethal concentrations of emetine.

## SUMMARY

1. A method, based on that devised for *E. histolytica* by Dobell (1947), was worked out for testing the action of drugs upon *E. invadens*.

2. The sensitivity of di- and monobacterial strains of *E. invadens* to emetine was determined, and of a dibacterial strain to the quinaldines A 352, A 356 and A 404. *E. invadens* was killed by emetine at concentrations of 1/50,000–1/100,000, A 352 or A 356 at 1/100,000, and A 404 at 1/10,000. The strains of *E. invadens* used in the present tests are thus less sensitive to emetine than is *E. histolytica*, but the sensitivity of the two species to the quinaldines is similar.

3. A strain of *E. invadens* which was maintained for 9 months in the presence of increasing concentrations of emetine, did not become resistant to the drug.

I am indebted to Dr A. Bishop for much advice and criticism; to Dr J. D. Fulton for a culture of *Bacterium coli* A′; and to Dr J. Walker for supplies of the compounds A 352, A 356 and A 404. The work has been financed by the Medical Research Council.

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# NEW BAT-FLEAS (SIPHONAPTERA: ISCHNOPSYLLIDAE)

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(With 32 Figures in the Text)

## The genus **Ischnopsyllus** Westwood

The genus *Ischnopsyllus* Westwood, comprising at the moment sixteen species and subspecies, is rather a heterogenous group. Although I am reluctant to split this relatively small genus, a certain degree of division is unavoidable. First, *I. grahami* Waterston certainly does not belong to the genus *Ischnopsyllus*, and since this species does not fit any other genus of bat-fleas, a new genus has had to be erected for it (see below, where a new subspecies of *grahami* is also described). Secondly, the *hexactenus*-group of *Ischnopsyllus* is sufficiently distinct from the rest of the genus to make it desirable to restore, as a subgenus, the genus *Hexactenopsylla* Oudemans, which was synonymized with *Ischnopsyllus* by Dalla Torre in 1924. What now remains in *Ischnopsyllus* is still heterogenous, but further division does not seem to be either necessary or desirable; the remaining species then fall into the following groups:

(*a*) *octactenus*-group, consisting of: *octactenus* (Kolenati), *consimilis* (Wahlgren), *variabilis* (Wagner), *simplex simplex* Rothschild, *simplex mysticus* Jordan, *hispanicus* Jordan, *dolosus* Dampf, *emminus* Jordan & Rothschild, and very likely also *elongatus* (Curtis), *intermedius* (Rothschild) and *plumatus* Ioff.

(*b*) *obscurus*-group, containing *obscurus* (Wagner) only.

(*c*) *needhami*-group, consisting of *needhami* Hsü and *liae* Jordan (a close relationship between these two species is not absolutely certain, but they appear to be closer related to each other than to any other species of the genus).

The two subgenera *Ischnopsyllus* and *Hexactenopsylla* can be separated as follows:

### Subgenus *Ischnopsyllus* s.str.

Metasternum without a squamulum (this may be present only as an extremely rare abnormality). *Male:* setae of main row of mesonotum never very long, the dorsal margin of the mesonotal collar not prolonged into a thin hair-like process. Apical half of sternum VIII not regularly spatulate. Anterior apical lobe of sternum IX broadish and truncate. Manubrium very broad, with a broadly rounded apex. Dorsal margin of aedeagal apodeme straight, apically not turned upward. Tendons of sternum IX making about half a convolution and those of the aedeagus about one whole convolution. *Female:* duct of spermatheca basally distinctly dilated, the transition into the unswollen portion abrupt. Type species: *Ceratophyllus elongatus* Curtis. This subgenus consists of the three species-groups mentioned above.

### Subgenus *Hexactenopsylla* Oudemans, 1909

Metasternum with a squamulum at or near the dorso-anterior corner. *Male:* setae of the main row of mesosternum usually very long and the dorsal margin of the mesonotal collar prolonged into a hair-like process (the long setae and hair-

like process are absent in *indicus* Jordan). Apical half of sternum VIII regularly spatulate. Anterior lobe of sternum IX ventrally with a rounded sinus, at the distal side of which there is a short cone bearing a seta. Manubrium very narrow. The aedeagal apodeme is strongly curved and has an upturned apex, therefore its dorsal margin is concave. Tendons of sternum IX and of aedeagus long, making at least one whole convolution, generally two. *Female:* duct of spermatheca basally not, or only slightly, dilated. Type species: *Ceratopsyllus hexactenus* Kolenati. To this subgenus also belong: *indicus* Jordan, *comans* Jordan & Rothschild, *delectabilis* Smit and *kolenatii* Wagner. The name of this subgenus is not very appropriate, since only *hexactenus* and *kolenatii* are six-combed fleas, the other species having eight combs, but inappropriateness of a name does not make its rejection possible.

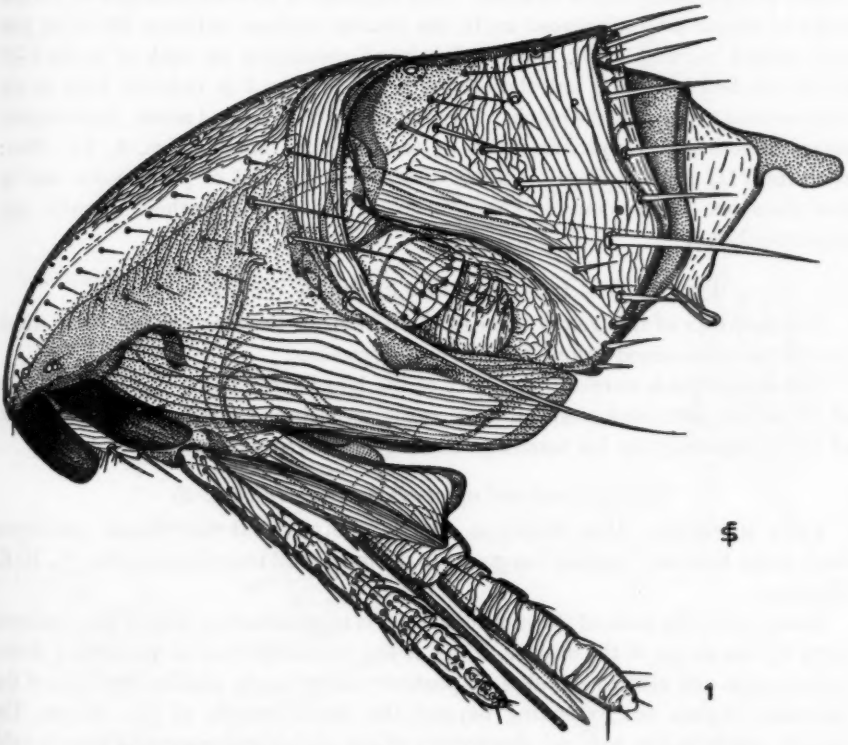


Fig. 1. *Dampfia grahami grahami* (Waterston). Head of female.

## **Dampfia**, n.g.

Related to *Oxyparius* Jordan, *Araeopsylla* Jordan & Rothschild and *Lagaropsylla* Jordan & Rothschild, but differing from these and all other genera of Ischnopsyllidae by the combination of the following characters:

Head (Fig. 1) without an arch of tentorium in front of the vestigial eye; frons long and sloping, with a hyaline submarginal band, between this band and the frontal margin a rugulose area; frons and occiput without conspicuous subdorsal

or dorsal incrassations. Spines of genal ctenidium both rounded apically, the anterior one being obtuse. Eye vestigial; ocular seta present above the eye. Frontal tubercle deciduous. Anterior membrane of labrum with many pseudosetae. Genal process not strongly sclerotized, broad. Stipes with a truncate apex. Pedicellus of antenna covering the basal third of the clava in the male, the basal half in the female. Tergal plates of thorax and abdomen without conspicuous dorsal incrassations. Mesonotum with a regular row of seven to nine pseudosetae each side under its collar, extending from the dorsal to the ventral margin of the collar (Fig. 26). Internal ridge of mesosternosome, between mesepisternum and mesosternum, incomplete and not reaching the anterior margin of the sternosome. Metathorax with a well-developed ctenidium; metasternum with a squamulum. Tibiae without false combs of setae. Fifth segment of all tarsi with one of the five pairs of lateral setae displaced on to the plantar surface, between the basal first and second pairs (Fig. 3). With a developed ctenidium on each of terga I–IV (a six-combed bat-flea), but the ctenidium of tergum I is reduced both in the number and the size of the spines. Sternum II without lateral setae. Antesensilial setae unmodified. Spiracular fossa of tergum VIII small (Figs. 4, 7). *Male:* sternum VIII not modified; tendons of sternum IX and of phallosome making less than one whole convolution. *Female:* duct of spermatheca basally only slightly dilated.

Type species: **Ischnopsyllus grahami** Waterston, 1915

The members of this genus occur in the Ethiopian Region; the few hosts which have been determined belong to the genus *Eptesicus*.

The new genus is named in memory of the late Dr Alfons Dampf, who, in spite of his rather restricted work on fleas, made important contributions in this field of study especially by his masterly drawings, hitherto unsurpassed.

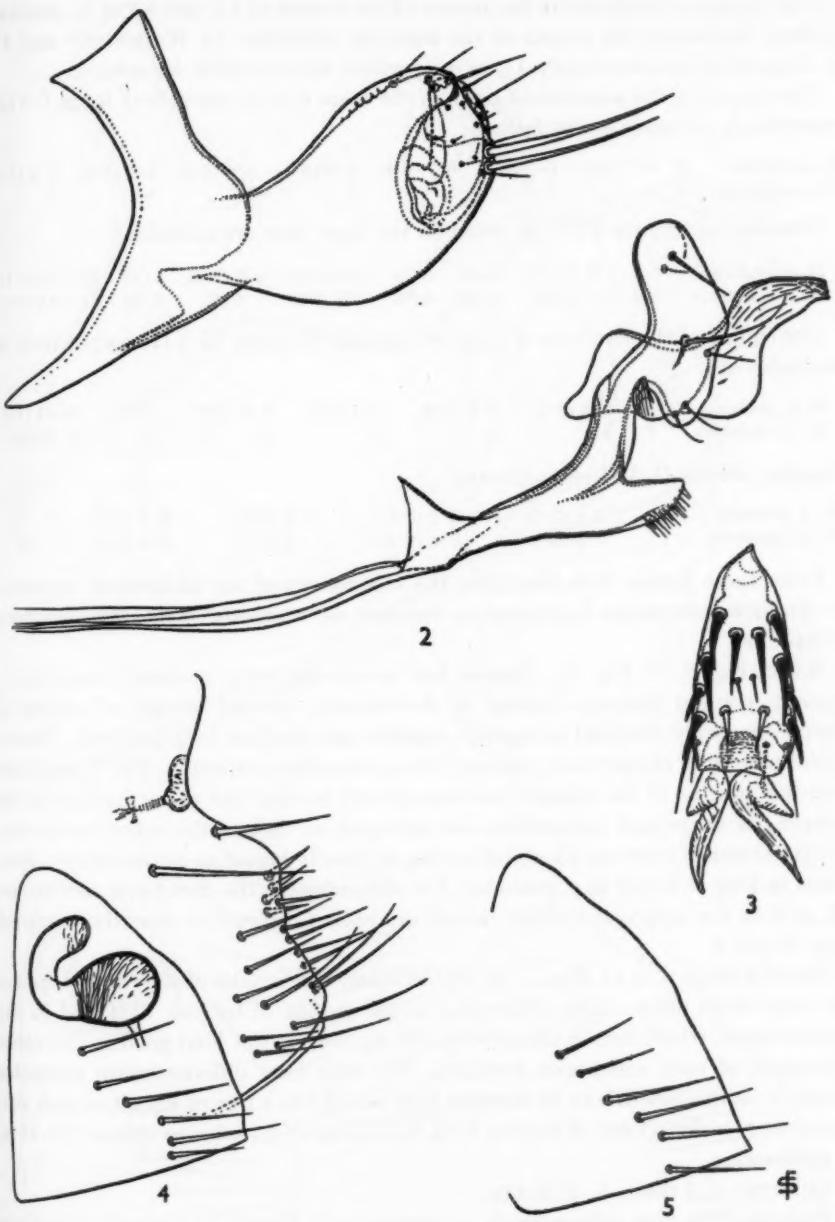
*Dampfia grahami equatoris* n.subsp. (Figs. 2–5)

TYPE MATERIAL. Male holotype, female allotype and two female paratypes from Kivu District, Belgian Congo, 1946, from a small insectivorous bat, G. H. E. Hopkins.

DIAGNOSIS. The male of the new subspecies is separable from that of the nominate form by the shape of the clasper, which is less broad and has no projecting dorso-apical angle and also no rounded-off postero-ventral angle, and by the apex of the movable process not extending beyond the dorsal margin of the clasper. The female differs in the reduced chaetotaxy of the abdominal segments (also a valid character for the male) and its sternum VII has a row of six lateral setae instead of seven as in the nominate subspecies.

DESCRIPTION. Apart from differences in chaetotaxy and in the male genitalia, the two subspecies resemble each other very closely. The counts of the numbers of spines in the ctenidia of pronotum, metanotum and terga I–IV respectively are as follows:

| | | | | | | |
|---|---|---|---|---|---|---|
| *D. g. grahami*, male | 26–28 (27·5) | 26–30 (28) | 7–8 (7·5) | 25–30 (27·5) | 22–24 (23·3) | 15–18 (16 |
| *D. g. equatoris*, male | 26 | 26 | 10 | 24 | 22 | 15 |
| *D. g. grahami*, female | 26–29 (28) | 26–31 (28·6) | 6–11 (9) | 24–28 (26·6) | 22–25 (23·8) | 15–18 (17 |
| *D. g. equatoris*, female | 27–29 (28) | 28(28) | 6–8 (7) | 26–28 (27) | 23–26 (24·3) | 19–20 (19 |




Figs. 2–5. *Dampfia grahami equatoris* n.ssp. 2, clasper, sternum IX and aedeagal crochet (holotype); 3, fifth hind tarsal segment (female paratype); 4, sternum VII, tergum VIII and spermatheca (allotype); 5, sternum VII (paratype).

The figures in brackets are the means of the counts of 4 ♂ and 6 ♀ of *D. grahami grahami* (including the counts of the holotype published by Waterston), and 3 ♀ of *D. grahami equatoris* (only 1 ♂ of *g. equatoris* was available for study).

The counts of the numbers of setae in the main row on one side of terga I–VIII respectively of males are as follows:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *D. g. grahami* | 4 | 6–7 (6·3) | 6–7 (6·3) | 6–7 (6·3) | 5–6 (5·3) | 5–6 (5·3) | 5–6 (5·2) | 7–8 (7·3) |
| *D. g. equatoris* | 4 | 6 | 5 | 5 | 5 | 5 | 5 | 7 |

Females (of tergum VIII the setae on the inner side are included):

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *D. g. grahami* | 4 | 6–7 (6·7) | 6 (6) | 6 (6) | 5–6 (5·8) | 5–6 (5·3) | 5 (5) | 25–31 (27·4) |
| *D. g. equatoris* | 4 | 6 (6) | 6 (6) | 6 (6) | 5–6 (5·3) | 5 (5) | 5 (5) | 21–23 (21·7) |

The counts of the numbers of setae on one side of sterna II–VIII respectively in the males are:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *D. g. grahami* | 1 | 2–3 (2·5) | 1–3 (2·3) | 2–3 (2·7) | 3–4 (3·2) | 3 (3) | 9–14 (11) |
| *D. g. equatoris* | 1 | 1 | 2 | 2 | 2 | 2 | 8–10 (9) |

Females, sterna II–VII respectively:

| | | | | | | |
|---|---|---|---|---|---|---|
| *D. g. grahami* | 1 | 3–4 (3·7) | 3–4 (3·5) | 3–5 (3·8) | 3–4 (3·8) | 7 |
| *D. g. equatoris* | 1 | 3 (3) | 3–4 (3·3) | 3 (3) | 3–4 (3·5) | 6 |

From these figures it is clear that the chaetotaxy of the abdominal segments in the new subspecies is somewhat reduced as compared with the nominate subspecies.

MALE (Fig. 2, cf. Fig. 6). Clasper less broad than in *g. grahami*; manubrium apically pointed upwards instead of downwards; ventral margin of corpus of clasper smoothly rounded as against angular and straight in *g. grahami*. Dorso-apical margin of clasper not produced into a nose-like projection. The apex of the movable process of the clasper does not extend beyond the upper margin of the clasper, its shape and proportions are subequal to that of the other subspecies. Proximal arm of sternum IX about as long as basally broad, as against about three times as long as broad in *g. grahami*. For differences in the distal arm of sternum IX and in the aedeagal crochet, which are rather difficult to describe, contrast Figs. 2 and 6.

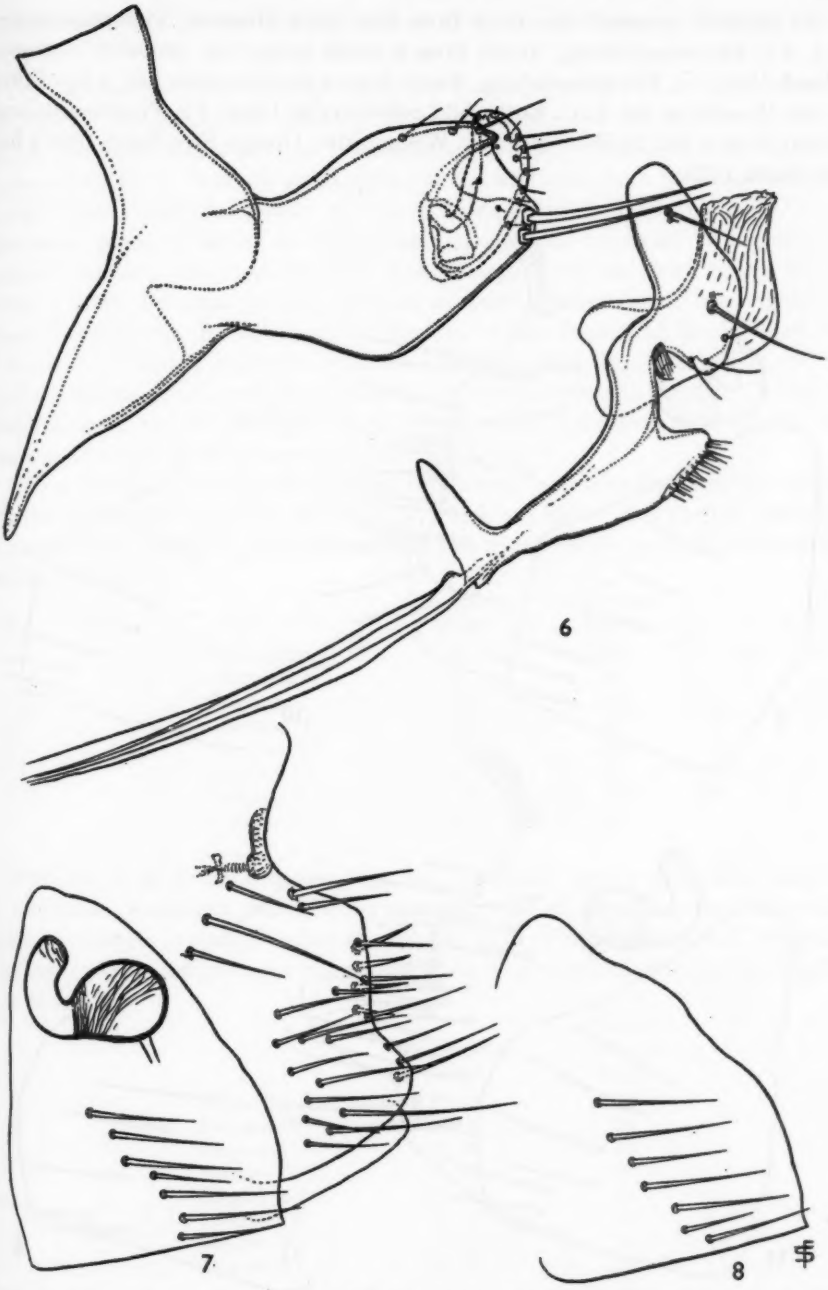
FEMALE (Figs. 4, 5, cf, Figs. 7, 8). Structurally the females of the two subspecies are very much alike; slight differences in the outline of tergum VIII and in the spermatheca, which can be observed in the figures, do not hold good in the other specimens of each subspecies available. The only easy differentiating character seems to be the chaetotaxy of sternum VII, which has a row of six setae each side (seven in *g. grahami*) and of tergum VIII (a total of 21–23 setae as against 25–31 in *g. grahami*).

LENGTH. ♂, 2 mm.; ♀, 2·25 mm.

REMARK. The new subspecies is at present only known to occur in the Kivu District of the Belgian Congo.

The following records of *D. grahami grahami* are known to me:

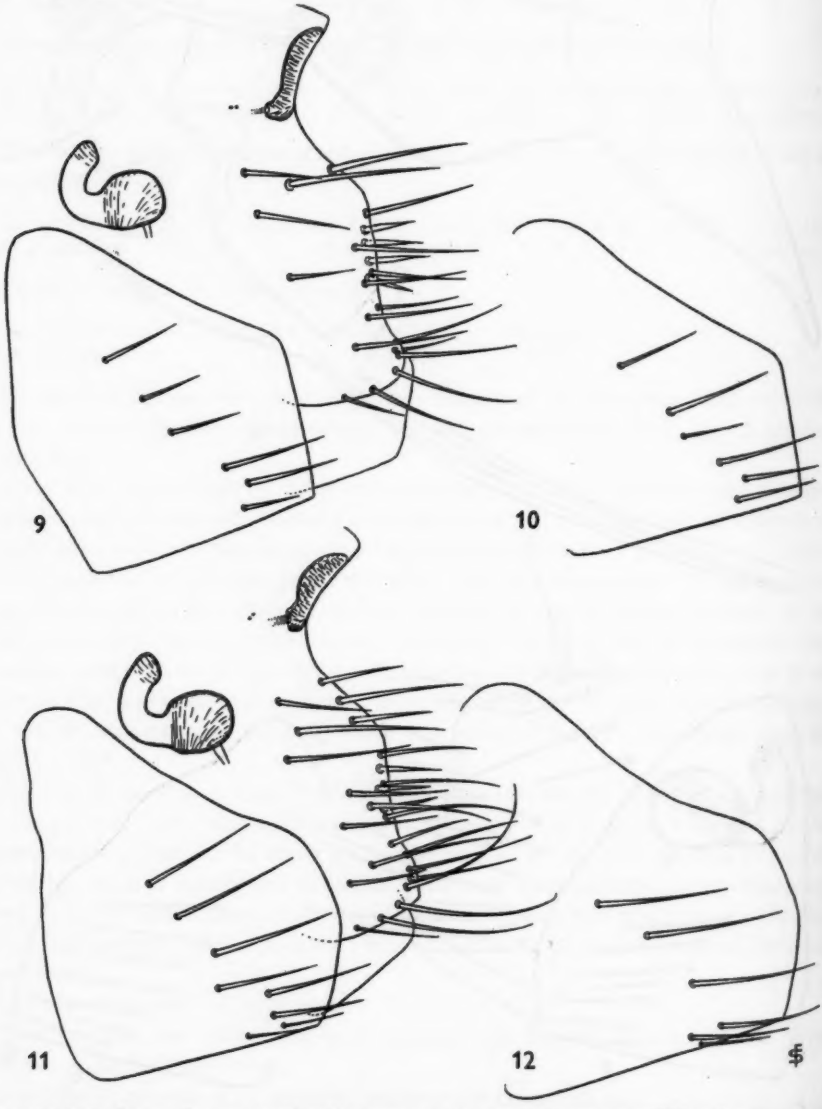
(*a*) Type material (Waterston, 1915): 1 ♂ (holotype), Grahamstown, Cape Province, from *Eptesicus capensis*, April 1913.




Figs. 6–8. *Dampfia grahami grahami* (Waterston). 6, clasper, sternum IX and aedeagal crochet (Pietermaritzburg); 7, sternum VII, tergum VIII and spermatheca (Pietermaritzburg); 8, sternum VII (Pietermaritzburg).

(*b*) Material received for study from the Natal Museum, Pietermaritzburg: 3♂, 3♀, Pietermaritzburg, Natal, from a small house bat, probably *Eptesicus*, March 1949; 1♀, Pietermaritzburg, Natal, from a small serotine bat, 1 April 1920.

(*c*) Material in the N. C. Rothschild collection at Tring: 1♀, Pietermaritzburg, Natal, from a bat, 22 Feb. 1918; 1♀, Westminster, Orange Free State, from a bat, 25 March 1925.




Figs. 9, 10. *Ischnopsyllus consimilis* (Wahlgren). 9, sternum VII, tergum VIII and spermatheca (Dagania, Israel); 10, sternum VII (Dagania, Israel). Figs. 11, 12. *I. octactenus* (Kolenati). 11, sternum VII, tergum VIII and spermatheca (Tring, England); 12, sternum VII (Tring).

REDESCRIPTION OF THE FEMALE OF *ISCHNOPSYLLUS CONSIMILIS* (WAHLGREN), 1904 (Figs. 9, 10)

I am indebted to Prof. O. Theodor of the Department of Parasitology, Hebrew University, Jerusalem, for allowing me to examine a few bat-fleas from Israel, which had been determined as *I. octactenus* (Kolenati). The fleas, 1♂, 2♀, were collected by Dr J. Palmoni at Dagania (on the southern shore of Lake Tiberias), Israel, from *Pipistrellus kuhli*. As was to be expected from their locality, these specimens prove to belong to the species *Ischnopsyllus consimilis* (Wahlgren), of which I recently described the male from a single specimen from Egypt (Smit, 1953, p. 206). I designated this specimen as neotype, since I have been unable to trace the holotype female. Dr Per Brinck, of the Zoological Institute of the University at Lund, Sweden, kindly informed me that this specimen is neither in his Institute, nor in any other likely Musea and Institutes in Sweden; Dr Brinck was informed by Dr Wahlgren (who described the specimen) that he did not remember where the specimen had been deposited.

The male of *I. consimilis* from Israel agrees very well with the neotype, except for the numbers of spines in the ctenidia, which are higher, but there is generally a considerable variation in these numbers. The counts of the spines in the ctenidia are as follows:

| | ♂ Dagania, Israel | ♂ Neotype, Egypt |
|---|---|---|
| Pronotum | 28 | 25 |
| Metanotum | 27 | 25 |
| Tergum I | 12 | 12 |
| Tergum II | 29 | 22 |
| Tergum III | 26 | 20 |
| Tergum IV | 21 | 17 |
| Tergum V | 15 | 13 |
| Tergum VI | 15 | 13 |

FEMALE (Figs. 9, 10, contrast Figs. 11, 12). Very closely resembling that of *I. octactenus* (Kolenati), and differing mainly by the shape of sternum VII, which posterior margin is straight below the marked angle of this margin, in *I. octactenus* the lower part of the posterior margin is convex. The counts of spines in the ctenidia are as follows:

| | 2♀ Dagania, Israel | Original ♀ holotype, Egypt |
|---|---|---|
| Pronotum | 27, 27 | 26 |
| Metanotum | 26, 28 | 24 |
| Tergum I | 12, 10 | 12 |
| Tergum II | 24, 23 | 20 |
| Tergum III | 20, 18 | 18 |
| Tergum IV | 18, 16 | 18 |
| Tergum V | 14, 11 | 14 |
| Tergum VI | 12, 10 | 12 |

Prof. Theodor generously presented one of the two females of *I. consimilis* from Israel to the British Museum collection of fleas at Tring.

*Thaumapsylla breviceps orientalis* n.subsp. (Fig. 13*a–d*)

*Thaumapsylla* '*breviceps* Roths.'—Jordan, 1937 (*nec* Rothschild, 1907), *Novit. zool.* **40**, 290 (Philippines).
*T.* '*breviceps* Roths.'—Jordan, 1939, *Novit. zool.* **41**, 374 (Burma).
*T.* '*breviceps* Rothschild'—Jordan, 1947, *Proc. Ent. Soc. Wash.* **49**, 184, fig. 2.
*T.* '*breviceps* Rothschild'—Traub, 1951, *Proc. Biol. Soc. Wash.* **64**, 18, figs. 26–31 (Philippines).

TYPE MATERIAL. Holotype male, allotype female and two male, three female paratypes from Jogeswary Caves, 7 miles from Bombay, India, from *Rousettus leschenaulti*, June 1952, L. S. Hiregaudar.

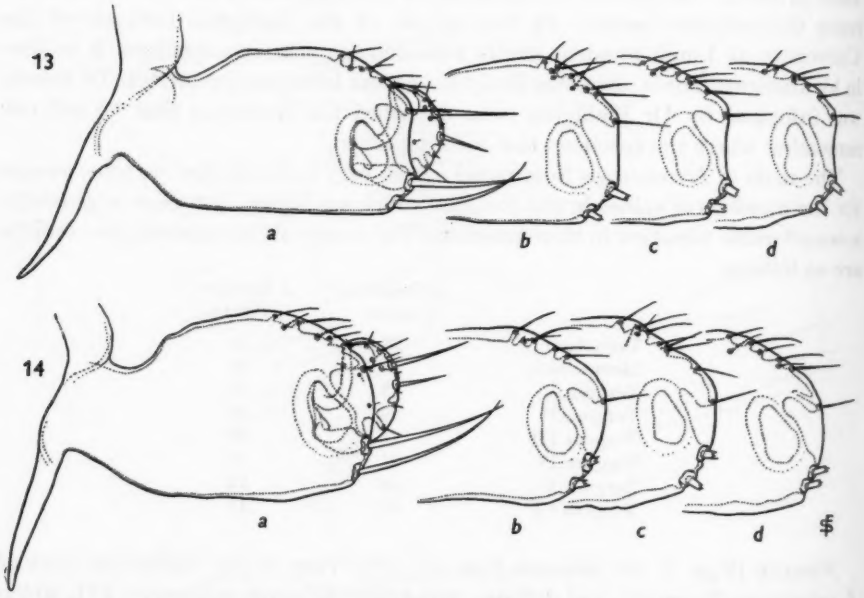



Fig. 13*a–d*. *Thaumapsylla breviceps orientalis* n.ssp. Claspers. *a*, from Jogeswary, India (holotype); *b*, from Tulungagung, Java; *c*, from Montalban, Luzon, Philippines; *d*, from Chiengdow Caves, Thailand. Fig. 14*a–d*. *T. breviceps breviceps* Rothschild. Claspers. *a*, from Zululand, Natal; *b*, from Knysna, Cape Province; *c*, from P. O. Marone, North Transvaal; *d*, from Kakontwe, Belgian Congo.

Paratypes: 2♂, 2♀, Rattota, Matale district, Ceylon, from *R. seminudus*, 21 November 1933, W. W. A. Phillips; 1♂, Gonagama, Kitulgala, Ceylon, from *R. seminudus*, 9 May 1925, W. W. A. Phillips; 1♂, Lamaing, 30 km. north of Mandalay, Burma, from *Rousettus* sp., G. Heinrich; 3♂, 3♀, Chiengdow Caves, Chiengmai, Thailand, from bats, 1914; 2♂, 3♀, Montalban, Province Rizal, Luzon Island, Philippines, from bat, E. H. Taylor per C. F. Baker; 1♂, 1♀, Kilibu Cave, Lubang Island, Philippines, from bat, received from J. Bequaert; 1♂, Tulungagung, Kediri, Java, from *R. amplexicaudatus*, 5 November 1932, C. J. Louwerens.

DIAGNOSIS. The new subspecies, occurring in the Oriental Region, is separable

from the Ethiopian nominate subspecies in the male by the posterior margin of the corpus of the clasper not being wholly convex, but concave (rarely straight). The females are practically indistinguishable.

DESCRIPTION. The average numbers of setae in the main row of the abdominal sterna are somewhat different in the two subspecies, but (since overlapping occurs), these counts cannot always be relied upon for purposes of identification. The means of the counts of a series of specimens are:

| | Sterna | | | | | |
|---|---|---|---|---|---|---|
| | III | IV | V | VI | VII | VIII |
| *T. b. breviceps*, male | 1 | 2·6 | 3 | 2·8 | 3·6 | 4·2 |
| *T. b. orientalis*, male | 1·8 | 3·4 | 3·6 | 3·8 | 4·2 | 4·5 |
| *T. b. breviceps*, female | 3·3 | 4·2 | 4·2 | 5·3 | 11·4 | — |
| *T. b. orientalis*, female | 3·5 | 4·3 | 4 | 4·3 | 9·6 | — |

MALE. The posterior margin of the corpus of the clasper is concave (Fig. 13*a*,*c*,*d*), seldom straight (Fig. 13*b*). In both subspecies the movable process is rather variable in shape and no reliable differences could be found between the two subspecies in this respect (see Figs. 13*a* and 14*a*). No structural differences between the females of the subspecies are apparent.

REMARK. Only the two following references in the literature apply to *Thaumapsylla breviceps breviceps* Rothschild: Rothschild, 1907, *Novit. zool.* **14**, 329, fig. 1 (Cape Colony); Cooreman, 1950, *Rev. zool. bot. afr.* **43**, 333, figs. 5–7 (Belgian Congo).

Of the nominate subspecies there are specimens in the Rothschild collection from: Cape Province (Cape Colony, Knysna, Pirie), and Natal (Zululand); I have also seen specimens from North Transvaal (P. O. Marone) and Belgian Congo (Kakontwe), through the courtesy of Dr B. De Meillon and Mr J. Cooreman respectively. The known distribution of *T. b. orientalis* is fully covered by the list of the type-material given above.

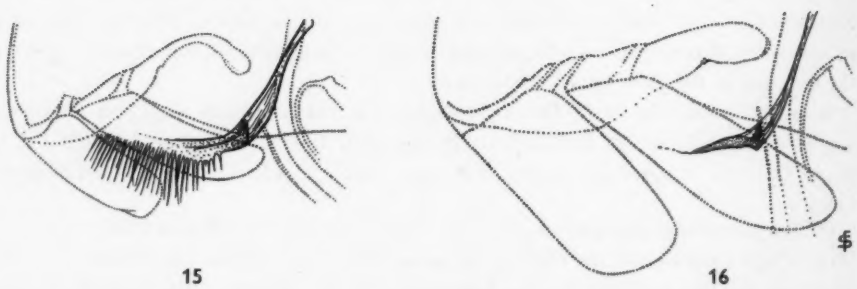
## SOME HITHERTO UNUSED DIAGNOSTIC CHARACTERS IN THE ISCHNOPSYLLIDAE (Figs. 15–32)

A general study of the Ischnopsyllidae resulted in detecting a few characters of generic value which had not been used as such up till now. They are:

(*a*) The presence or absence of an arch of the tentorium on each side of the head in front of the vestigial eye. This tentorial arch is present in: *Thaumapsylla* Rothschild, *Sternopsylla* Jordan & Rothschild, *Myodopsylla* Jordan & Rothschild, *Ischnopsyllus* Westwood, *Oxyparius* Jordan, *Rhinolophopsylla* Oudemans. The tentorial arch is absent in: *Ptilopsylla* Jordan & Rothschild, *Hormopsylla* Jordan & Rothschild, *Nycteridopsylla* Oudemans, *Araeopsylla* Jordan & Rothschild, *Lagaropsylla* Jordan & Rothschild, *Dampfia* Smit, *Chiropteropsylla* Oudemans and *Porribius* Jordan. It is presumably also absent in *Rothschildopsylla* Guimarães, which I have not seen but which appears to be closely related to *Ptilopsylla* and *Hormopsylla*. It should be noted that in some specimens of species of genera in which the tentorial arch is absent, an extremely small and thin vestige of the arch

may be present on one or both sides of the pre-antennal part of the head, visible only by careful observation under high power; for practical purposes it is reasonable to ignore such vestiges.

(*b*) The labrum is usually situated between the posterior spine of the genal ctenidium and its fellow on the opposite side of the head, and therefore is at some distance from the anterior wall of the cranium, to which it is connected by a longish membrane. This membrane, and, to a certain extent, also part of the labrum itself, may bear numerous slender pseudosetae (Fig. 15) (easily visible in mounted specimens, between the genal spines ; see Fig. 1) or may bear none at all (Fig. 16). In the following genera the membrane possesses numerous pseudosetae: *Ischnopsyllus*, *Myodopsylla*, *Oxyparius*, *Araeopsylla*, *Lagaropsylla*, *Chiropteropsylla* and *Dampfia*. The pseudosetae of the labral membrane are absent in: *Rhinolophopsylla*, *Porribius*, *Nycteridopsylla*, *Sternopsylla*, *Ptilopsylla*, *Hormopsylla* (probably *Rothschildopsylla*, since it is stated to be close to the two preceding genera) and *Thaumapsylla*.



15 16

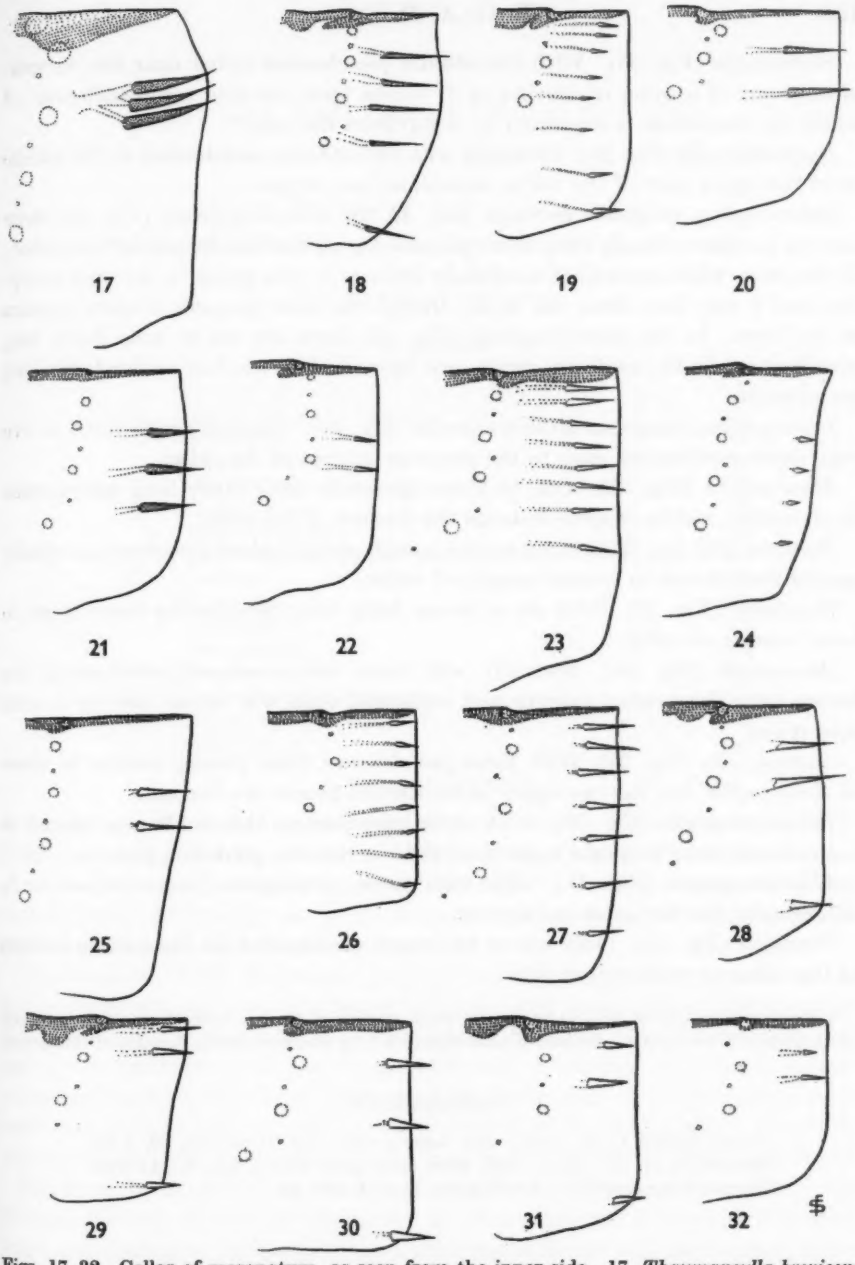
Figs. 15, 16. Preoral part of head, to show the labrum. 15, *Dampfia grahami grahami* (Waterston); 16, *Rhinolophopsylla unipectinata unipectinata* (Taschenberg).

(*c*) The number and placing of the pseudosetae under the collar of the mesonotum has proved to be of greater generic importance than is generally accepted. Although there is commonly some intra-generic variation in the numbers of these pseudosetae (the variation becoming greater as the numbers increase), each genus has a certain, rather stable number, and moreover the arrangement of the pseudosetae is frequently peculiar to the genus. Examples of the mesonotal pseudosetae, as seen from the inside of the collar, in all genera of Ischnopsyllidae except *Rothschildopsylla* are shown in Figs. 17–32. Here follow some notes on the numbers and their variability in each genus.

*Thaumapsylla* (Fig. 17). In 75 % of the specimens examined there are two stout pseudosetae on one or (more commonly) both sides of the collar, in the remaining 25 % there are three pseudosetae.

*Ptilopsylla* (Fig. 18). Four or five slender pseudosetae, the last (lowermost) being separated by a gap from the penultimate one.

*Hormopsylla* (Fig. 19). Four or five small and short pseudosetae in *H. fosteri*, six in *H. trux* and seven or eight in *H. egena*; in the first two species the lowermost pseudoseta is separated from the penultimate one by a gap.




Figs. 17–32. Collar of mesonotum, as seen from the inner side. 17, *Thaumapsylla breviceps breviceps* Rothschild; 18, *Ptilopsylla dunni* Kohls; 19, *Hormopsylla egena* Jordan; 20, *Sternopsylla texana* (C. Fox); 21, *Nycteridopsylla eusarca* Dampf; 22, *Ischnopsyllus* (*Ischnopsyllus*) *octactenus* (Kolenati); 23, *I.* (*Ischnopsyllus*) *obscurus* (Wagner); 24, *I.* (*Hexactenopsylla*) *hexactenus* (Kolenati); 25, *Myodopsylla insignis* (Rothschild); 26, *Dampfia grahami grahami* (Waterston); 27, *Oxyparius isomalus* (Waterston); 28, *Araeopsylla martialis* (Rothschild); 29, *Lagaropsylla incerta* (Rothschild); 30, *Rhinolophopsylla unipectinata unipectinata* (Taschenberg); 31, *Chiropteropsylla brockmani* Rothschild; 32, *Porribius caminae* (Rothschild).

*Sternopsylla* (Fig. 20). With two slender pseudosetae rather near the dorsum; in only one of a series of females of *S. texana* there are four setae each side, of which the lowermost is separated by a gap from the rest.*

*Nycteridopsylla* (Fig. 21). Generally with two or three pseudosetae at the middle or at the upper part of the collar, sometimes one or four.

*Ischnopsyllus*, subgenus *Ischnopsyllus*. In the *octactenus*-group (Fig. 22) there are one to three (usually two) short pseudosetae in the middle part of the collar; *I. elongatus*, which somewhat doubtfully belongs to this group, is the only exception and it may have from one to six, though the more frequent number appears to be three. In the *obscurus*-group (Fig. 23) there are six to nine fairly long pseudosetae. In the *needhami*-group (not figured) there are four or five fairly long pseudosetae.

*Ischnopsyllus*, subgenus *Hexactenopsylla* (Fig. 24). Generally with three to five very short pseudosetae close to the posterior margin of the collar.

*Myodopsylla* (Fig. 25). One to three (generally two) fairly long pseudosetae in about the middle or more towards the dorsum of the collar.

*Dampfia* (Fig. 26). With seven to nine spindle-shaped, short pseudosetae, equally spaced from dorsal to ventral margin of collar.

*Oxyparius* (Fig. 27). With six or seven fairly long pseudosetae from upper to lower margin of collar.

*Araeopsylla* (Fig. 28). Normally with three well-developed pseudosetae, the lowest near the ventral margin and separated from the upper two by a wide interspace.

*Lagaropsylla* (Fig. 29). With three pseudosetae, their placing similar to those of *Araeopsylla*, but the two upper ones situated nearer the dorsum.

*Rhinolophopsylla* (Fig. 30). With three pseudosetae, the middle one placed at a greater distance from the uppermost than in the two preceding genera.

*Chiropteropsylla* (Fig. 31). Also with three pseudosetae, more or less as in *Araeopsylla*, but the setae are shorter.

*Porribius* (Fig. 32). Only one or two short pseudosetae on the middle portion of the collar or on its upper half.

* This was thought at first to be an abnormal specimen, since it lacks the anal stylets, but I subsequently found that absence of anal stylets is very common in the females of this genus.

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# ON BAT PARASITES IN PALESTINE

## I. NYCTERIBIIDAE, STREBLIDAE, HEMIPTERA, SIPHONAPTERA

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(With 105 Figures in the Text)

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### I. INTRODUCTION

In connexion with studies on bat malaria carried out by Prof. G. Mer and Dr N. Goldblum at the Malaria Research Station of the Hebrew University, a great number of ectoparasites of bats were submitted to us for identification. Together with material collected by us, over a thousand nycteribiids, streblids, Hemiptera, Siphonaptera, ticks and mites were examined.

Examination of the Palestinian material showed that the systematics of the family Nycteribiidae were in a rather unsatisfactory state, and we had therefore to extend our study to related species outside Palestine. The present paper, in basing the systematics of the Nycteribiidae on a number of hitherto unused characters, particularly of the structure of the thorax and of the genitalia in both sexes, attempts to provide material for a revision of the family which, of course, will have to be based on far more species than were available for the present study.

We have to thank the authorities of the British Museum (Nat. Hist.), the Chicago Natural History Museum, the Muséum National d'Histoire Naturelle, Paris, the Zoological Institute of the University of Berlin and the German Entomological Institute, the Zoological Museum in Vienna, the Zoological Museum in Genoa, and the Zoological Museum in Amsterdam for putting their material at our disposal. Most of the drawings were made by Mr N. Gratz.

The ticks and mites parasitic on bats in Palestine will be dealt with in another paper.

## II. THE BAT FAUNA OF PALESTINE

The following list is based on records by local collectors, on the *Check-list of Palaearctic and Indian Mammals* by Ellerman & Morrison-Scott (1951), and on *Studies on Bats and Bat Parasites* by Ryberg (1947). We have to thank Dr M. Dor, who has made the bats of Palestine his special study, for information on the occurrence of bats and their biology. We have also to thank Dr N. Goldblum for permission to quote observations on bats and bat parasites from his Ph.D. thesis (Hebrew University, Jerusalem, 1951).

Inclusion of a species in the list is not intended to establish a record of the occurrence of this species in Palestine. A number of questions concerning the identity of species has still to be cleared up, and in some cases it is not known whether the record refers to the typical or another subspecies.

### MEGACHIROPTERA

#### PTEROPIDAE

1. *Rousettus aegyptiacus* Geoffroy, 1810.

Distribution: Ethiopian Africa in part, south to Angola, Egypt, Palestine, Syria, Cyprus.

### MICROCHIROPTERA

#### RHINOPOMATIDAE

2. *Rhinopoma microphyllum* Brünnich, 1782.

Arabia, Egypt, Palestine, perhaps Persia, Morocco.

3. *R. hardwickei arabium* Thomas, 1913.

Arabia, Palestine, Morocco.

#### EMBALLONURIDAE

4. *Taphozous nudiventris* Cretzschmar, 1830.

Arabia, Egypt, Palestine, Sudan, Morocco.

#### NYCTERIDAE

5. *Nycteris thebaica* Geoffroy, 1818.

Arabia, Egypt, Palestine, Sudan, Kenya, Angola, Corfu (not recorded by local collectors).

#### RHINOLOPHIDAE

6. *Rhinolophus ferrumequinum* Schreber, 1774.

Europe, Algeria, Morocco, Palestine, Asia.

7. *R. hipposideros minimus* Heuglin, 1861.

Mediterranean, Arabia, Palestine, East Africa.

8. *R. euryale judaicus* Andersen & Matschie, 1904.

Egypt, Palestine.

9. *R. blasii* Peters, 1866.

Syn. *R. clivosus* Blasius, 1857 (nec Cretzschmar, 1828).

Mediterranean, Palestine, West Asia, Arabia.

10. *Asellia tridens* Geoffroy, 1813.
East Africa, Morocco, Arabia, Egypt, Palestine, Iraq, Persia, India.

MOLOSSIDAE

11. *Tadarida teniotis* Rafinesque, 1814.
Mediterranean, Morocco, Palestine, Egypt, West Asia.

VESPERTILIONIDAE

12. *Myotis mystacinus* Kuhl, 1819.

Europe, Asia, Palestine (?). According to Ellerman & Morrison-Scott this species has not been recorded south of the Caucasus in the Western Palaearctic. Ryberg records it for Syria and Palestine as a doubtful record on map 18 and as reliable occurrence in table 4.

13. *M. emarginatus* Geoffroy, 1861.
Europe, North-west Africa (Ryberg), Palestine (?). Records of this species from Palestine probably refer to *M. nattereri*.

14. *M. nattereri* Kuhl, 1818.
Europe, Palestine, Asia.

15. *Myotis myotis* Borkhausen, 1797.
Syn. *Vespertilio murinus* Schreber, 1774 (nec Linné, 1758).
Europe, Palestine, Asia, Morocco.

16. *M. blythi oxygnathus* Mont., 1885.
Europe, North-west Africa, Palestine, South-west Asia.

17. *M. daubentoni* Kuhl, 1819.
Europe, Asia. Ryberg records it as doubtful from Palestine on map 24. Records from Palestine probably refer to *M. capaccinii*.

18. *M. capaccinii* Bonaparte, 1837.
South Europe, North Africa, Palestine.

19. *Eptesicus serotinus* Schreber, 1774.
Europe, West Asia, North Africa, Arabia, Palestine, Syria, Anatolia (Ryberg).

20. *Nyctalus noctula* Schreber, 1774.
Europe, North-west Africa, Asia, Palestine. (Ryberg, doubtful record, map 38.)

21. *Pipistrellus nathusii* Keyserling & Blasius, 1839.
Europe, Palestine. (Ryberg, doubtful record, map 30.)

22. *Pipistrellus kuhli* Kuhl, 1819.
Europe, Africa, West Asia, Palestine, Morocco.

23. *Otonycteris hemprichi* Peters, 1859.
North Africa, Egypt, Palestine, West Asia.

24. *Plecotus auritus christiei* Gray, 1838.
Egypt, Sudan, Palestine, Tunis.

25. *Miniopterus schreibersi* Kuhl, 1819.
Europe, Algeria, Morocco, Palestine.

Tristram's record of *Scotophilus temmincki* Horsfield, an oriental species, is certainly incorrect. Of the above list, *Rousettus aegyptiacus*, *Rhinopoma microphyllum*, *Taphozous nudiventris*, *Nycteris thebaica* and *Asellia tridens* are Ethiopian and extend into the Western Palaearctic in Palestine and Syria. A number of other species (*Rhinolophus blasii*, *R. euryale*, *Tadarida teniotis*, *Otonycteris hemprichi*) have a mainly Mediterranean distribution, extending eastwards into Asia. Most other species are widely distributed in Europe and Palaearctic Asia, and many of them reach their southern limit of distribution in the western parts of the Palaearctic region in Palestine. *Plecotus auritus* shows a similar general distribution but penetrates along the Nile and along the east coast of Africa southwards to Eritrea. The Nile Valley and the East African Rift Valley, of which the Red Sea forms the connecting link between its southern part and its continuation into Asia, the Jordan Valley, form one of the great migration routes along which bats, as well as many other animals, have migrated, as shown by the occurrence of the Ethiopian species mentioned above. These bats have brought with them parasites which also belong to the Ethiopian fauna. Only ten of the twenty-five species or subspecies of the above list occur in Egypt. Of these the five species mentioned above are Ethiopian and extend through Egypt into Palestine, while the other five are Mediterranean. No species of the genera *Myotis* and *Miniopterus* have been recorded from Egypt which, as will be shown below, has a marked influence on the constitution of the parasite fauna of Egyptian bats.

Relatively little is known about the actual migration of bats in our area. *Rousettus aegyptiacus* is present in the country all the year round and is found in winter in great numbers in caves in the coastal area (Herzliah, Ramleh). Similarly, *Rhinopoma microphyllum* and *R. hardwickei arabium* have been found to form a permanent colony in a cave near Tiberias. They were found in numbers from January onwards. The bats were sluggish on cold days, but they were not hibernating. They were easily awakened and able to fly in January. *Asellia tridens* appears in spring and disappears again in autumn, but no exact information is available as to how far individual bats migrate. The movements of some colonies of *Miniopterus schreibersi* have been observed by Goldblum, who examined a number of caves regularly throughout the year. *M. schreibersi* was present in small numbers in winter in a cave in southern Palestine, appeared in May–June in great numbers in a cave near Mount Carmel and moved from there to a cave in northern Galilee in August and September. From here the bats disappeared at the beginning of winter, apparently on their way south. Similarly, other species appeared in late spring in a cave and disappeared from the country in autumn. Most species of bats were found only in small numbers during winter, and they apparently do not hibernate regularly in the country. *Rhinolophus ferrumequinum*, however, has been observed to hibernate in Palestine.

There are some interesting observations by Goldblum on the association of the various species, which may have a bearing on their parasite fauna. Various species of *Myotis* and *Miniopterus schreibersi* roost in large mixed colonies while the Rhinolophidae either roost in separate colonies or singly. There seems to be a definite antagonism between *Myotis myotis* and species of the genus *Rhino-*

*lophus*. Thus all the *Rhinolophus* were observed to leave a cave in May when *Myotis myotis* began to appear there. *Pipistrellus kuhli* inhabits not caves but houses and roofs and has a parasite fauna which differs sharply from that of other species which live in caves.

## III. NYCTERIBIIDAE

### Nomenclature

The early nomenclature of the Nycteribiidae is in a very confused state, mainly due to the fact that authors used to call nearly every parasite found on a bat 'vespertilionis'. Collins (1931) discussed the nomenclatural questions in detail and from his fully documented paper the following facts appear well established.

Linné (1758) named '*Pediculus vespertilionis*', basing it on a description and illustration by Frisch (1728). This figure, which has been reproduced by Collins, clearly represents a mite of the genus *Spinturnix* or *Ancystropus*. The characteristic position of the legs, four pointing forward and four backward, is unmistakable. In addition, Linné states: 'Acaro aut Phalangio simile.'

In 1796 Latreille proposed a new genus, *Nycteribia*, giving as type species *Acarus vespertilionis* Linn. Fab. Pediculus, Linn. His diagnosis, quoted by Collins, leaves no doubt, however, that he was dealing with an insect. He states: 'Tarses longs, articulés, terminés par deux longs crochets. . . .Corcelet séparé de l'abdomen, déprimé, portant six pattes longues.' Latreille therefore was in error in creating a genus for an insect and giving an acarid as type species. Apparently, as Collins says, he did not consult the original paper of Frisch on which Linné based his *Pediculus vespertilionis*. This is thus 'a case in which the type species cited for a genus is based upon an erroneous determination of a species' (Collins).

It has been suggested by Collins that either the name *Nycteribia* should be validated in its present sense or that both names, *Nycteribia* and *Pediculus vespertilionis*, should be suppressed. It seems unreasonable, however, to sink the name *Nycteribia*, which has been in constant use for over 150 years and is the basis for the generally recognized family name Nycteribiidae. Handlirsch (1925) has accordingly included both the generic and the family name in his list of *nomina conservanda*.

In 1805, Latreille corrected his error by redefining *Nycteribia* and proposing a new specific name for *N. vespertilionis*: *N. pedicularia*. The illustration given is clearly that of a nycteribiid. His diagnosis, however, is not sufficiently clear to allow the recognition of the species he was dealing with. He uses the terms: 'Cuisses et jambes très comprimés, presque elliptiques', which would suggest a species of *Listropoda* (*sensu* Kolenati), but it is impossible to decide which of the four European species of *Listropoda* he had before him, since the type specimen is apparently lost.

In 1804 Hermann created a new genus, *Phthiridium*, with *Phthiridium vespertilionis* as type species. This insect has been recognized by Westwood (1835) as distinct from the other two British species (*N. kolenatii* nom.nov. for *N. blasii* Kolenati, 1863 (*N. latreillii* according to Westwood) and *N. biarticulata* Herm.) and has been renamed by him: *N. vexata*. The elongated form of the tibiae, their

length as compared with the femora and the segmentation of the abdomen can be reasonably interpreted from Hermann's figure as belonging to *N. vexata* as we understand it to-day. Westwood's suggestion that it may possibly be identical with Latreille's *Nycteribia pedicularia* may therefore be disregarded since Hermann's figure does not agree with Latreille's description. Hermann's second species *Phthiridium biarticulatum* is clearly recognizable from his figure.

Montagu (1808) mentions *Celeripes vespertilionis* without giving any description. This is thus a *nomen nudum*. In 1815 he illustrates and describes *Nycteribia vespertilionis* and cites *Celeripes vespertilionis* as synonym. Thus, as Collins states: 'While *vespertilionis* of 1808 is a *nomen nudum*, *vespertilionis* (*Celeripes*) of 1815 is a dead homonym of *vespertilionis* of Latreille, 1796, in case it is construed as a distinct species.' This is so in fact, as Montagu's figure clearly shows the cylindrical processes at the tip of the abdomen characteristic for *Nycteribia biarticulata*, and these processes are expressly mentioned and clearly described in the text. *N. vespertilionis* Montagu thus becomes a synonym of *N. biarticulata*.

Leach (1817) describes and illustrates *Phthiridium hermanni* which is identical with *Nycteribia biarticulata* and thus becomes a synonym of the latter. He also renames *N. pedicularia* Latreille, *N. latreillii*. The name *N. latreillii* would therefore become a synonym of *N. pedicularia* if this were a single well-defined species. As pointed out above, however, it is a composite species containing possibly three or four species of *Listropoda* (*sensu* Kolenati). The further fate of the name *latreillii* will be discussed in connexion with Kolenati's (1863) species of this group.

Westwood (1835) recognizes five European species, *Nycteribia dufourii*, *N. vexata*, *N. pedicularia*, *N. latreillii* and *N. biarticulata*. Of these *N. dufourii* and *N. biarticulata* are clearly defined. *N. vexata* was recognized by Westwood as different from what he called *N. latreillii* and may be considered as identical with *N. vexata* of to-day. In 1840, Westwood created a new genus, *Stylidia* for *Nycteribia biarticulata*. In 1856, 1857, and in more detail in 1863, Kolenati described several additional species, among them *Listropoda latreillii*, *L. blasii*, and redescribed *L. schmidlii* Schiner, 1853, but did not redescribe *Nycteribia pedicularia* which he thought (1857) possibly identical with his *N. frauenfeldi* (= *P. dufourii*). In 1863 he changed his view and cited *N. pedicularia* with a question mark in the synonymy of *Listropoda latreillii*. As pointed out above, *Nycteribia pedicularia* Latreille is probably a composite species consisting of what is called to-day *N. pedicularia*, *N. latreillii*, *N. kolenatii* (= *N. blasii* Kol. 1863) and *N. schmidlii*. One may therefore assume that Kolenati restricted the species by redefining three out of the four species possibly contained in *N. pedicularia* Latr., thus leaving the fourth species, *N. pedicularia*, as we understand it to-day. In 1856 Kolenati described *N. blasii* as a new species, but his description leaves no doubt that the species described was in fact *N. schmidlii* Schiner. He corrected this in his paper of 1863, where he described *N. schmidlii* correctly, but now named another species *N. blasii*. This is inadmissible, and the name *N. blasii* Kol. 1856 must be considered as a synonym of *N. schmidlii*. The species named *N. blasii* in 1863 has therefore to be renamed and the name *N. kolenatii* is proposed. Further details are given below.

In 1857 and 1863 Kolenati subdivided the Nycteribiidae into a number of genera,

among them *Acrocholidia*, *Stylidia* and *Listropoda*. The name *Nycteribia* was used only as a general term for species, the generic position of which was still doubtful. There is no reason given for the change of name of the genus *Nycteribia*, and as his genus *Listropoda* contains the type species of the genus *Nycteribia* (*N. pedicularia* Latr.), *Listropoda* becomes a synonym of *Nycteribia*.

In 1901 Speiser re-introduced the genus *Nycteribia* and subdivided it into three subgenera, *Acrocholidia*, *Stylidia* and *Listropoda*. As this last name is a synonym of *Nycteribia* and as the subgenus containing the type species has to have the same name as the genus (Art. 9, Int. Rul. Nom.) the subgenus *Listropoda* also becomes a synonym of *Nycteribia*.

Musgrave (1925) discusses the above subgeneric names of the genus *Nycteribia*, and arrives at conclusions at variance with the views expressed above. As pointed out above, Latreille's name *N. pedicularia* (1805) should be maintained as the correction of an error and because of its generally accepted use. Reasons have also been given, why *N. vexata* Westw. should not be regarded as identical with *N. pedicularia*. On the other hand, there are reasons to assume that one or more of the species of *Listropoda* Kol. are in fact *Nycteribia pedicularia*. The subgeneric name *Listropoda* and not, as Musgrave suggests, *Acrocholidia*, should therefore be considered as synonym of *Nycteribia*. Nor can we follow Musgrave in his reasoning for the preservation of the name *Celeripes* for Hermann's species *Phthiridium biarticulatum*. As pointed out by Musgrave himself, this was a *nomen nudum* in 1808 and Montagu's citing of this name in synonymy with his description of *Nycteribia vespertilionis* in 1815 does not, as Musgrave thinks, establish its validity, but makes it, as Collins says, 'a dead homonym of *N. vespertilionis* Latr.'. The generic (or subgeneric) name *Stylidia* Westwood 1840 is thus the first designation of a generic name for *Nycteribia biarticulata* and is therefore valid.

It is obviously impossible to decide with certainty some of the nomenclatorial questions discussed above, but the suggestions made here seem the most reasonable in the circumstances, involving the minimum of changes.

## Morphology

The principal characters used hitherto in describing genera and species of Nycteribiidae are as follows: presence or absence of thoracic and abdominal ctenidia and the form and number of the teeth in them. Presence or absence of eyes, their structure and pigmentation. The shape and structure of the legs, particularly of the tibiae, the form of the head and of the abdominal sclerites and chaetotaxy. All these characters are of either generic, subgeneric or specific value. The value of some characters, however, seems to have been overrated. Too much stress has been laid in the past on minor variations in chaetotaxy. The number, shape and position of hairs is very variable and descriptions based on single specimens are thus often misleading.

The head of the Nycteribiidae has a number of characters of systematic value. The absence or presence of eyes is a character of generic value. The structure of the eye, whether single or double, the structure of the ocular sclerite and its pigmentation afford further characters. The head of some species is broadly rounded

(*Penicillidia*), in others it is more or less compressed laterally, particularly anteriorly where the anterior margin is often folded into an angle. In *Cyclopodia* the head is flattened dorso-ventrally. The dorsal surface of the head is either wholly sclerotized up to the anterior margin, or the anterior median part is soft and membranous. The chaetotaxy of the head is characteristic in the genus *Penicillidia* and *Cyclopodia*, but more or less uniform in *Nycteribia*, *Basilia* and *Eucampsipoda*, except for minor details (Fig. 1*a–c*).

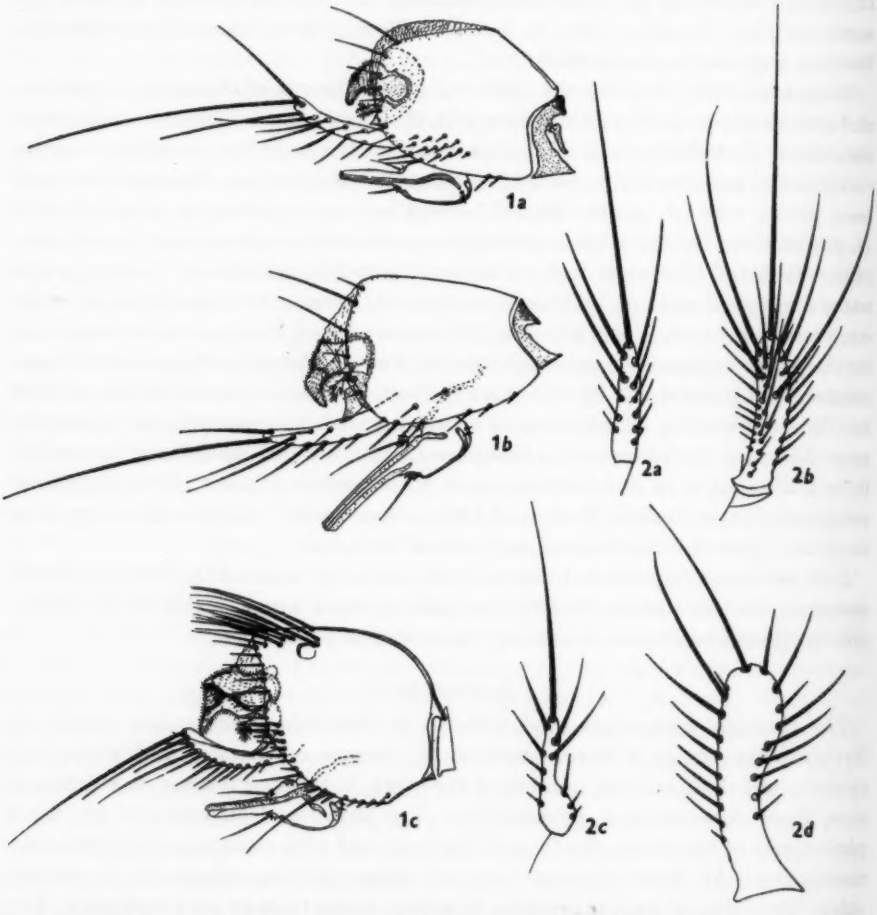



Fig. 1. Head of: *a*, *Nycteribia latreillii*; *b*, *N. vexata*; *c*, *Penicillidia dufourii*. Fig. 2. Palps of *a*, *Nycteribia latreillii*; *b*, *Penicillidia dufourii*; *c*, *Eucampsipoda hyrtlii*; *d*, *Cyclopodia sykesii*.

The palps afford a number of generic characters. In most species of the genera *Nycteribia* and *Basilia* they are finger-shaped, slightly flattened dorso-ventrally, with a very long seta at the tip and two rows of 4 to 8 hairs along both edges of their ventral surface. These hairs are shorter near the base and longer or very long near the tip, but never as long as the apical seta. In *Penicillidia* the palps are

thicker with several dense rows of long and short setae in addition to the terminal seta which, however, is not so much longer than the others as in *Nycteribia* and *Basilia*. In *Eucampsipoda* the palps are thick at the base and very thin anteriorly with only 5 to 6 hairs in addition to the long terminal seta and there are a few short hairs at the base. In *Cyclopodia* the palps are flattened and bear two rows of short hairs along the edges and a longer seta at the tip (Fig. 2*a–d*).

The proboscis of *Cyclopodia sykesii* has been described in detail by Jobling (1928). The proboscis of the other genera is similar in structure but its proportions and chaetotaxy differ markedly. The basal bulb, the theca, is about as long or slightly longer or shorter than the anterior narrow part, the labella, in the genera *Penicillidia* and *Nycteribia* (Fig. 1). In the genus *Basilia* the labella are distinctly shorter than the theca, in one species about a third of its length. In the genus *Eucampsipoda* the labella are nearly twice as long as the theca (Fig. 78), and in *Cyclopodia* their length varies. In some species of this genus the labella are as long as the theca, but in others they are markedly longer. The chaetotaxy of the proboscis is relatively uniform in the genera *Nycteribia*, *Basilia* and *Penicillidia*. There are two longer setae ventrally on each side near the junction of the theca and the labella and a few small hairs near them. Near the posterior edge of the theca there are two minute spines on each side. There are three such spines in *Eucampsipoda* and 4 to 5 in some species of *Cyclopodia*.

*Thorax*. The shape of the thorax differs in the various genera. Thus in *Eucampsipoda* (Fig. 79) the thorax is rather narrow anteriorly, between the ctenidia, while in *Penicillidia* and *Nycteribia* it is much wider. In the genus *Penicillidia* the thorax is markedly wider than long, while in other genera the relation between length and width varies. Length and width of the thorax should be measured at the strongly sclerotized ventral surface.

A character of great systematic value is the pattern on the dorsal side of the thorax (Fig. 3), formed by the notopleural sutures in which the anterior spiracles are situated, the mesopleural sutures which run from the notopleural sutures laterally and end in front of coxa 2, the sclerotized area adjoining the notopleural sutures laterally and the notopleural hairs on this area. The area between the notopleural sutures represents the mesonotum and its anterior part, on which the head and forelegs are situated, the prothorax. The areas lateral to the notopleural sutures represent the pleurae. In *Cyclopodia* and *Eucampsipoda* the notopleural sutures lie close together anteriorly and diverge posteriorly. The spiracles are situated at about the anterior third and the mesopleural suture originates at the posterior third, bearing one or two hairs in *Eucampsipoda* and 1, 2 or 3 hairs in different species of *Cyclopodia*. In *Penicillidia* the spiracles lie further anteriorly and have a slit-shaped opening. The notopleural sutures lie farther apart, converging posteriorly and the mesopleural suture is rather long and leaves the notopleural suture much further anteriorly than in *Eucampsipoda*. The number of notopleural hairs in *Penicillidia* varies from 7 to 8 in some species, to 1 or 2 in other species and in some species the notopleural hairs are absent.

On the whole, the species of *Nycteribia* and *Basilia* have a pattern similar to that of *Penicillidia*, except for minor differences, which, however, are useful in

differentiating species. *Nycteribia biarticulata*, *N. biloba* n.sp. and *N. integra* n.nom. are very similar in their dorsal thoracic pattern and in the wider spacing of the anterior notopleural hairs.

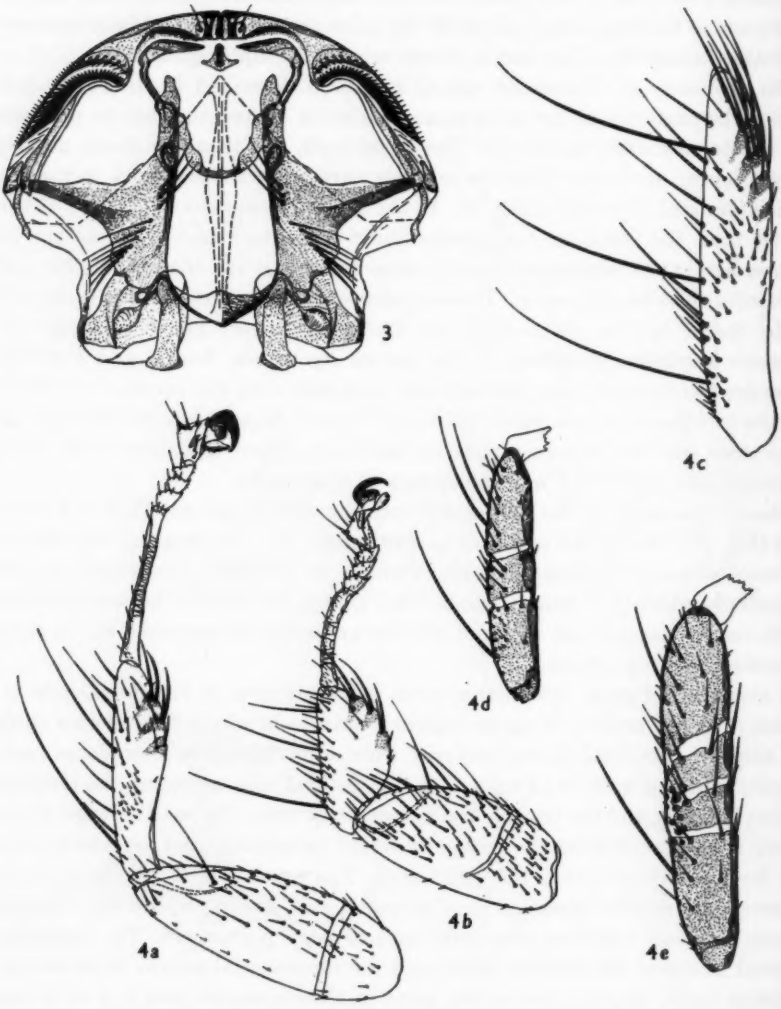



**Fig. 3.** Thorax of *Nycteribia latreillii*, dorsal side. The dotted lines indicate the sutures of the ventral side. **Fig. 4.** Fore-legs of: *a*, *N. biarticulata*; *b*, *N. latreillii*. Fore-tibiae of: *c*, *Penicillidia dufourii*; *d*, *Eucamposipoda hyrtlii*; *e*, *Cyclopodia greeffi*.

The halteres and the posterior spiracles lie in deep grooves at the sides of the notopleural sutures near the posterior margin of the thorax. In all eight species of *Penicillidia* examined these grooves are covered by a broad flap which originates at the posterior margin of the thorax and is free anteriorly (Fig. 70). This flap has been interpreted in the past as the haltere itself by some authors. Except for

*Penicillidia* this flap has so far been found to be present only in three oriental species of *Cyclopodia* (*C. sykesii*, *C. horsfieldi* and *C. inclita*). It is lacking in other species of *Cyclopodia* examined (*C. greeffi*, *C. dubia* and *C. ferrarii*). It is also present in *Eremoctenia*, according to the drawing given by Scott (1914), who, however, considered the cover as the haltere itself. In all the other genera (*Eucampsipoda*, *Nycteribia*, *Basilia*, *Tripselia*, *Paracyclopodia* and *Archinycteribia*) the cover is either lacking or rudimentary.

There is no correlation between the presence and development of the notopleural hairs and the presence or absence of the haltere groove cover. The notopleural hairs probably function as a protective fan over the haltere groove, being bent backwards when the insect moves through the fur. In all species of *Penicillidia* the haltere groove cover is present, but the row of notopleural hairs is well developed in some species, reduced to 1 to 3 hairs in others, and absent in some species. In *Cyclopodia* there are 1 to 3 notopleural hairs in species where the cover is present and the same number of hairs is found in species without the cover. In *Eucampsipoda* there are only 1 or 2 hairs and the haltere groove is uncovered.

The ventral pattern of the thorax also shows some characters of systematic value. The median sternal suture is either narrow or widened in parts, and the sutures between meso- and metasternum (Winkelleisten, called oblique sutures for brevity in this paper) which join the median suture anteriorly differ in the angle at which they join the median suture. These sutures and the median sutures are indicated by dotted lines in the drawings of the dorsal thoraxic patterns. The angle mentioned in the descriptions refers to the angle between the two lateral sutures and not to the angle between the lateral suture and the median suture. In some genera (*Penicillidia*) the sternal plates are firmly joined and the lateral sutures are therefore indistinct, while in others they are formed by a strip of thinner chitin and are thus clearly marked. The ventral hind margin of the thorax bears a row of setae which shows some characters of systematic value. In *Eucampsipoda* and *Cyclopodia* the setae are short in the middle, growing slightly longer laterally. In the genera *Nycteribia*, *Basilia* and *Penicillidia* there are in addition to the short setae one or two long setae on the lobes at each side of the median concavity. In the subgenus *Stylidia* the number is reduced to 1 long and 4 to 5 short setae at each side, leaving a wide gap in the middle. In the South African *Nycteribia scissa* there is only one short hair at each side.

The thoracic and abdominal ctenidia consist of specialized, flattened spines with secondary striations. The teeth forming these ctenidia are large and have blunt tips in *Eucampsipoda* and *Cyclopodia*. In all other genera examined they are much narrower and pointed. In some species of *Penicillidia* the abdominal ctenidium consists of these specialized spines which stand close together. In other species of the genus the ctenidium consists of ordinary spines which are present also in other parts of the body. They are much more widely spaced and sometimes reduced in size so that one may say that there is no real ctenidium present. The genera *Archinycteribia* and *Eremoctenia* are based on the absence of the abdominal or of the abdominal and thoracic ctenidia. Although the absence of the thoracic ctenidium is certainly a character of generic value, the absence of the abdominal

ctenidium in itself does not seem to be sufficient for the characterization of a genus in view of the position in the genus *Penicillidia*.

The presence of rings on the tibiae has been used as one of the main characters for the definition of genera (*Tripselia*, *Cyclopodia*). All Nycteribiidae have several transverse rows of setae on the tibiae, and the chitin distally to these rows is as a rule thinner than the area on which the setae stand, thus forming more or less distinct rings. In some genera the rings are not easily visible because they are covered by the numerous long, thick setae and extend only partly round the tibia (*Penicillidia*, *Nycteribia*). In others the rings are more marked, the chitin forming them is thinner, the setae near them are few and short, and the rings are situated in the middle of the tibia, surrounding it completely. These rings are especially well marked in the genus *Cyclopodia*, where there are three and in *Eucampsipoda* with two. There are three such rings, however, in the genera *Paracyclopodia* and *Tripselia*, both of which show more affinity to the genus *Basilia* than to *Cyclopodia* (Fig. 4*a–e*).

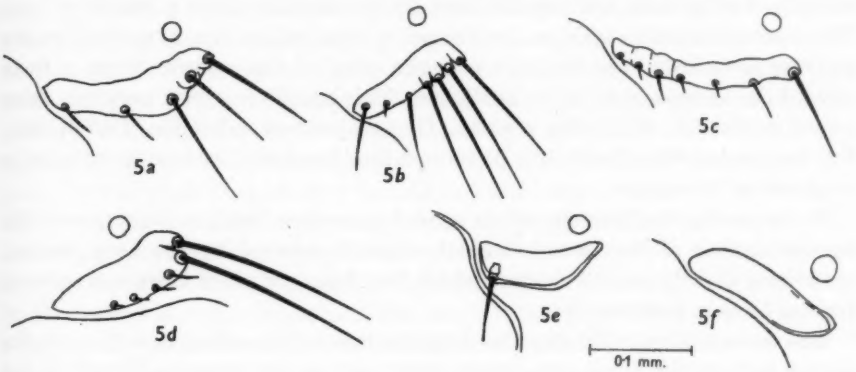
The abdomen in the Nycteribiidae is formed of six segments, not taking into account the segments which form the genitalia. Several of these segments are sometimes fused into broad plates or into a connecting membrane, particularly in the female. The male abdomen has preserved its primitive segmentation to a much higher degree. Tergites 1 to 6 are usually distinct and tergite 7 and following are included in the anal segment as shown by the presence of spiracle no. 7 inside the anal segment. In *Eucampsipoda* and *Cyclopodia* and in a few species in other genera, tergites 1 and 2 are completely fused. The first two sternites are fused in all Nycteribiidae, male and female, and this plate bears the abdominal ctenidium in all genera except the two tropical genera, *Archinycteribia* and *Eremoctenia*, in which it is replaced by a row of thin hairs. A segment of particular systematic value is the fifth sternite of the male, which in most species bears a characteristic armature of spines. In some genera (*Eucampsipoda*, *Basilia*) there is a small characteristic sclerite ventral to the anus of the female which has proved useful in distinguishing the species.

A structure of generic value is the post-spiracular sclerite (Fig. 5). This is a small, narrow, strip-like plate which lies at the anterior lateral corner of sternites 1 and 2 and reaches to a point a short distance behind the first spiracle. This sclerite is difficult to see as it lies beneath the hind coxa in alcohol specimens and is generally seen foreshortened in balsam preparations.

In the genera *Nycteribia* and *Basilia* it is a narrow, curved band, which bears several long setae at the posterior margin near the free side, towards the spiracle and shorter setae towards sternite 1+2. The length and number of the setae and the form of the plate is somewhat variable within the species, but it has a characteristic shape and chaetotaxy in some species. In the genus *Paracyclopodia* the sclerite is rather broad and triangular and bears 2 to 4 strong setae at the end near the spiracle. In *Eucampsipoda* the sclerite is rather broad and short with a single seta near sternite 1+2. In *Cyclopodia* it is of similar form but without any setae and in *Penicillidia* the sclerite is entirely absent.

### Genitalia

The structure of the genitalia in the Nycteribiidae has been very little studied so far. Falcoz (1926) gives an illustration of the genitalia of *Nycteribia schmidlii*. Cole (1927) gives a ventral view of the genital area of *N. pedicularia* and *N. biarticulata*, but does not describe its structure in detail. Karaman (1936 to 1948) describes and illustrates aedeagus, parameres and claspers of a number of species



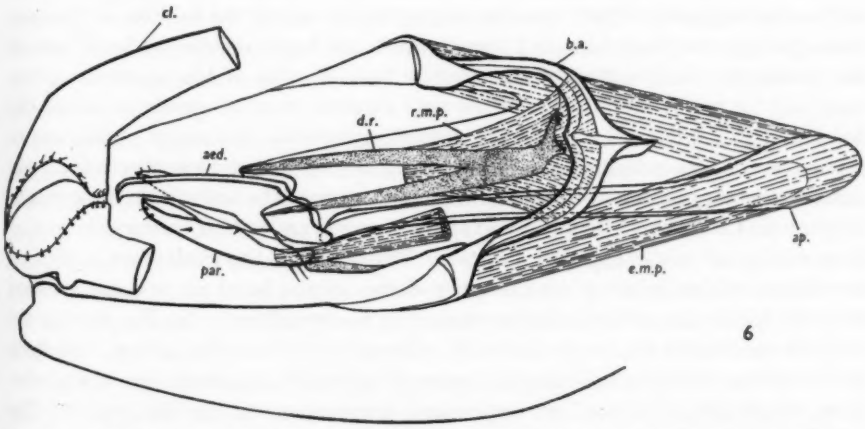




Fig. 5. Postspiracular sclerites of: *a*, *Nycteribia latreillii*; *b*, *N. biarticulata*; *c*, *Basilia nattereri*; *d*, *B. bathybothyra daganiae*; *e*, *Eucampsipoda hyrtlii*; *f*, *Cyclopodia greeffi*. Fig. 6. Male genitalia of *Nycteribia latreillii* (semi-diagrammatic, half-profile). *aed.* aedeagus; *ap.* apodeme of aedeagus; *b.a.* basal arc; *cl.* clasper; *d.r.* dorsal ridge of phallobase; *e.m.p.* erector muscle of phallobase; *r.m.p.* retractor muscle of phallobase; *par.* paramere.

and mentions the genital plates of the female, without, however, giving a complete description of the genital apparatus. Hennig (1941) gives a diagrammatic drawing of the male genitalia of *Penicillidia jenynsii* and Corradetti & Lupascu (1941) describe and illustrate those of some Italian species.

The male genitalia of most Nycteribiidae are of a very simple and generalized structure (Figs. 6, 21). The aedeagus consists of two lateral plates fused ventrally,

forming a trough closed above by a thin membrane with a wide longitudinal opening into which the ductus ejaculatorius opens. The aedeagus articulates posteriorly with a long, laterally flattened apodeme. The aedeagus lies inside a conical phallobase which bears two parameres distally. These parameres can be turned back around their basal articulations in most species. In the resting position the parameres lie alongside the aedeagus and form a sheath around it. The phallobase is strengthened along its median anterior line by a pigmented sclerotized ridge with a triangular base which generally bears a few short hairs. This base articulates with an arc-shaped sclerite which has a median process pointing anteriorly. The concave posterior edge of this sclerite forms a frame around the anterior side of the phallobase. This sclerite has been variously interpreted as the 7th, 8th or 9th sternite. German authors call it the 'Gabelplatte'. For the present this sclerite will be termed the 'basal arc' without attempting to establish its homology.

In the resting position the whole genital apparatus lies in a depression of the ventral surface of the last abdominal segment, covered by the long, pointed, sometimes slightly curved claspers which bear long and short hairs and spines of various form in some species.

Muscles extend from the tip of the long apodeme of the aedeagus to the posterior lateral flaps of the basal arc, which forms part of the lateral edge of the last abdominal segment. Other muscles originating at about the middle of the apodeme go into the phallobase and some strands are inserted into the basal part of the paramere. Contraction of the muscles from the tip of the apodeme to the basal arc (or to the lateral edge of the anal segment in some species in which the basal arc does not reach far enough posteriorly) causes the whole phallobase to revolve around its anterior basal hinge, so that it stands at right angles to the body surface. By contraction of the abdomen and pressure of the body fluid the aedeagus is protruded for a short distance and the parameres are turned backwards so that they now point in the opposite direction. Retraction of the phallobase is effected by muscles which originate on the inner surface of the basal arc and are inserted into the basal part of the anterior surface of the phallobase. In Fig. 6 only the muscles mentioned above are shown in order not to obscure the picture, but there are a number of additional muscles, some going from the apodeme into the phallobase, others from the basal arc to the anal segment and to the fifth sternite. The function of these muscles has not yet been fully worked out.

A modification of this type of genitalia which may prove of subgeneric value is present in the subgenus *Stylidia*. Apart from *Nycteribia biarticulata*, similar genitalia have been found in several species which according to the old classification were placed in the subgenus *Acrocholidia* (*Nycteribia biloba*, *N. integra*, *N. scissa* and *N. hindlei*), as they do not possess the abdominal processes characteristic for the female of *N. biarticulata*. In these species the parameres have a characteristic shape with a long apical process and they are partially or completely fused with the phallobase. The aedeagus is also of a similar form in all these species.

The vas deferens runs at the beginning ventrally to the hind intestine, then

ascends at its left side, traverses the intestine dorsally, descends at its right side and enters the base of the aedeagus. There is thus a hypopygium circumversum which is characteristic for all higher Diptera.

In the genus *Eucampsipoda* the genital apparatus has become more complicated. The parameres are reduced to small triangular plates closing the phallobase in front of the aedeagus which in the resting position lies wholly retracted within the phallobase. The aedeagus has the form of a wide tube with an oblique anterior opening. Its dorsal wall consists of a thin membrane, the anterior part of which is deeply invaginated into the aedeagus and bears a great number of small teeth. This membrane is everted during copulation, forming a large bulbous endophallus, in the centre of which the gonopore lies. The aedeagus is connected at about its middle with the anterior edge of the phallobase by a membrane which bears a large number of spines on its ventral surface. During copulation three actions take place: (1) The phallobase revolves around its basal hinge through 90° as described above. (2) The aedeagus is protruded from the phallobase by the length of the connecting membrane and its teeth are everted so that they now point backwards and presumably aid in holding the aedeagus in position in the uterus of the female. (3) The endophallus is everted from the anterior opening of the aedeagus, forming a large vesicle covered with numerous backwards pointing teeth. At the base of its funnel-shaped opening lies the gonopore. Protrusion of the endophallus and the aedeagus is effected by contraction of the abdomen and the resulting pressure of the body fluid. The parameres are connected by a V-shaped ridge. During protrusion of the aedeagus the parameres are folded back over the anterior side of the phallobase (Fig. 7*a*, *b*).

The genital apparatus of *Eucampsipoda* thus shows two additional features as compared with that of other Nycteribiidae, an endophallus and a connective membrane which allows a much greater protrusion of the aedeagus than the other type of genitalia described previously. It is quite impossible to arrive at an understanding of this mechanism from the study of preserved material. Examination of fresh material in which the movements of the various membranes can be effected by exerting pressure on the abdomen, dissection of fresh material and histological sections are necessary.

In the genus *Cyclopodia* a similar development, in some respects more extreme, has taken place. The phallobase has a strongly sclerotized anterior dorsal plate which ends distally in a hooked process (Fig. 8*b*). The parameres are reduced to small triangular plates as in *Eucampsipoda*, closing the phallobase in front in the resting position. The whole genital apparatus lies in the resting position invaginated in a pouch, deep within the abdomen, well behind the phallobase, reaching into the second or third abdominal segment (Fig. 8*a*). This pouch has been observed by Speiser (1901) who thought that it may be 'a recess of the ductus ejaculatorius'. The aedeagus consists of a membranous tube, in the wall of which lies a sclerotized rod, tapering distally. At its base this rod bears secondary arms in some species, giving it the shape of an anchor. The distal part of the aedeagus is evaginated until the sclerotized rod is fully exposed anteriorly and the small teeth of the pouch are everted and point backwards as in the connecting membrane in

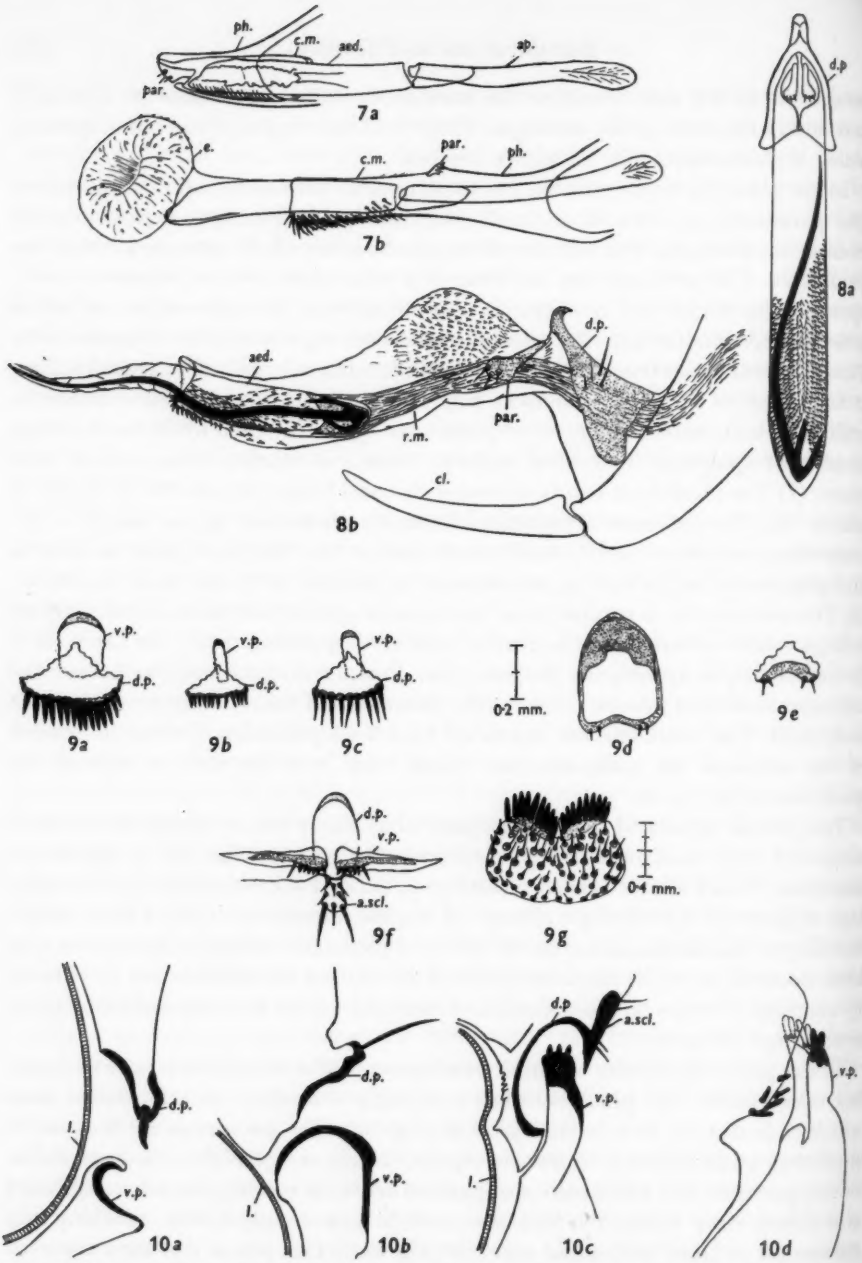



Fig. 7. Male genitalia of *Eucampsipoda hyrtlii*: *a*, resting position; *b*, extended position; *aed.* aedeagus; *ap.* apodeme; *c.m.* connecting membrane; *e.* endophallus; *ph.* phallobase; *par.* paramere. Fig. 8. Male genitalia of *Cyclopodia greeffi*: *a*, resting position; *b*, nearly extended position; *aed.* aedeagus; *cl.* clasper; *d.p.* dorsal plate of phallobase; *par.* paramere; *r.m.* retractor muscle of aedeagus. Fig. 9. Genital plates of females of Nycteribiidae: *a, Nycteribia latreillii*; *b, N. pedicularia*; *c, N. kolenatii*; *d, N. biarticulata*; *e. N. vexata*; *f, Eucampsipoda hyrtlii*; *g, Cyclopodia greeffi*. *a.scl.* anal sclerite; *d.p.* dorsal genital plate; *v.p.* ventral genital plate. Fig. 10. Median sagittal sections through end of abdomen of female Nycteribiidae: *a, Nycteribia latreillii*; *b, Penicillidia dufourii*; *c, Eucampsipoda hyrtlii*; *d, Cyclopodia sykesii*. *l.* larva. Other letters as in Fig. 9.

*Eucampsipoda*. The basal part of the pouch forms a wide bulge also partly covered with small teeth which point backwards. A long retractor muscle is attached to the base of the aedeagus. Fig. 8*b* shows the situation before evagination is completed. The sclerotized rod reaches to the tip of the aedeagus in the species figured (*Cyclopodia greeffi*), in other species the rod does not reach to the tip of the aedeagus and the distal end of the aedeagus seems to form a small endophallus. Protrusion of the whole apparatus apparently takes place by compression of the abdomen and pressure of the body fluid and retraction by action of the muscle inserted into the base of the aedeagus. The origin of the retractor muscle and the course of the ductus ejaculatorius could not be established because of lack of fresh material and of material fixed for histological sections.

The hook at the tip of the phallobase probably assumes the function of intromittent organ and the aedeagus is everted after the introduction of the point of the phallobase into the genital opening of the female. The phallobase with its hook has been incorrectly interpreted as penis by Karaman (1939).

The type of genital apparatus described above has been found so far in *C. sykesii*, *C. greeffi*, *C. dubia*, *C. horsfieldi*, *C. inclita* and *C. similis* (*fide* Speiser). In *C. ferrarii* and *C. macrura*, which according to the structure of head, thorax and legs doubtless belong to this genus, a type of genitalia similar in some respects to that of the genus *Nycteribia* has been found. A revision of the genus according to the structure of the genitalia may therefore eventually become necessary.

The shape of the aedeagus and parameres vary considerably in the different species in all genera, but most of these differences seem to be of specific rank. Only in the subgenus *Stylidia* is the shape of parameres and aedeagus characteristic for all species, as far as they have been studied, as well as the fact that the parameres are fused with the phallobase to a varying degree.*

*Female genitalia*. The genital plates of the female have also not been used so far as systematic characters. They are mentioned casually by Speiser, Corradetti and Karaman. Examination of these plates has shown, however, that they permit the differentiation of closely related species, e.g. of the subgenus *Nycteribia* which are otherwise difficult to separate. In the genera *Cyclopodia* and *Eucampsipoda* these plates are especially well developed in a form characteristic for the genus.

It is difficult to make out the relative position of these plates in alcohol-preserved material, except in specimens in which the abdomen is greatly distended by the fully developed larva. In balsam mounts which as a rule are compressed dorso-ventrally, the plates appear to lie in the same plane. By cutting the abdomen of a female in the median sagittal plane and mounting the cleared specimen with the

* A further study of the male genital apparatus, particularly of the genus *Cyclopodia* is intended and the writer (O.T.) would be grateful for material fixed for histology either in Bouin's fluid for 24 hr. and preserved in 70 % alcohol or in Kahle's fluid (alcohol 96 %, 17 parts; formalin 40 %, 6 parts; glac. acetic acid, 2 parts; aqua dest. 28 parts) for 24 hr. and preserved in 70 % alcohol. If possible, some specimens should be fixed with genitalia everted, by compressing the abdomen and holding the insect thus in the fixative for a few minutes. Specimens kept in glycerine, 1 part, and alcohol 70 %, 1 part, remain pliable and permit the study of the genitalia nearly as well as in fresh material.

median side upwards a clear picture of the relationship of the various structures can be obtained (Figs. 10, 75*b*).

In most species examined there are two genital plates, a dorsal and a ventral. The ventral plate apparently represents sternite 7 or 8. In some species of the genera *Nycteribia* and *Penicillidia* the dorsal plate is roughly triangular and bears a row of long or short spines at its posterior margin (Fig. 9*a–c*). These spines cover the genital opening from above. The plate itself lies inside the genital opening, forming part of its dorsal wall. In *Penicillidia conspicua* the plate is fused with the postanal sclerite, so that it appears double-walled (Fig. 75*a*, *b*). The ventral plate bears no teeth in these genera. It varies in form in the different species, but in general it shows a rounded, more heavily chitinized anterior border and an irregular posterior border. In some species its posterior surface is convex (*P. conspicua*, *P. dufourii*) (Figs. 10*b* and 75*b*), in others it is concave (*Nycteribia latreillii*, Fig. 10*a*).

In *N. biarticulata* and *N. biloba* only the dorsal genital plate is present. It has a rounded anterior border in both species and is devoid of spines except for a few minute hairs. It is rather small in *N. biarticulata* and larger in *N. biloba* (Figs. 9*d* and 39*a*, *b*). In *N. vexata* there is also only a dorsal plate in the form of a short arc concave posteriorly with 4 to 6 short spines (Fig. 9*e*). In some species the dorsal plates are much reduced (*Basilia bathybothyra* and *B. nana*) and the ventral plates are absent or very small.

In the genus *Eucampsipoda* the plates are well developed in a form characteristic for the genus (Fig. 9*f*). The ventral plate is divided into two wing-shaped halves which at their dorsal edge bear a number of short spines. The shape of the plates and the number and arrangement of the teeth vary in the different species. The dorsal plate is strongly concave posteriorly and rounded anteriorly. The plate is fused with a small postanal sclerite which bears a characteristic set of hairs and spines in different species. The sagittal section (Fig. 10*c*) shows that the teeth of the ventral plates project into the lumen of the genital opening. The dorsal plate forming the vaulted roof of the genital opening from the inside and the postanal sclerite from the outside form a lip which covers the genital opening from above in the same manner as the toothed plate in the species of *Nycteribia*. The dorsal vaulted plate apparently prevents pressure on the projecting teeth of the ventral plate. The dorsal plate covers the teeth of the ventral plates in some species, so that they are not visible from the outside, except in greatly distended specimens.

In the genus *Cyclopodia*, there is a somewhat similar arrangement. There is only a ventral genital plate which bears a row of spines covering the genital opening from the ventral side (Fig. 9*g*). There is no dorsal plate, the dorsal lip of the genital opening being formed by the postanal sclerite which in several species bears a group of spines. These lie anteriorly to the row of spines on the genital plate (Fig. 10*d*). In other species examined this group of teeth is absent.

The arrangement of the plates described above is no doubt of generic character in *Eucampsipoda*. In *Cyclopodia* the arrangement described is present in the six species named above, but in *C. ferrarii* and *C. macrura* the arrangement is different. As pointed out above, these species also differ in the structure of the male genitalia.

In a species of the new genus *Dipseliopoda*, which will be described shortly in another paper, a type of genitalia intermediate between those of *Eucampsipoda* and *Cyclopodia*, is present. In general outline, the genitalia resemble those of *Eucampsipoda*, but the endophallus is divided into two large dorsal and two small ventral lobes and continues into a long tapering tube similar to the aedeagus of *Cyclopodia*. Only the ventral genital plate is present in the female and resembles that of *Cyclopodia*. In another species of the same genus the aedeagus has become very long and thin and there is no endophallus.

There are thus three or four types of male genitalia and three corresponding arrangements of genital plates in the female in the family which apparently are of generic character.

## Systematic part

Diagnoses of genera and descriptions of many species are either contained in old papers—the last comprehensive study of the family was written by Speiser in 1901—or are scattered in journals which are often not easily accessible. Provisional definitions of some genera and descriptions of species are therefore given, with the addition of a number of characters not used previously, mainly concerning the dorsal pattern of the thorax and the structure of the male and female genitalia.

### Genus **Nycteribia** Latreille, 1796

Type species: *Nycteribia pedicularia* Latreille, 1805.

This genus is perhaps the least well defined of all. It has been characterized mainly on negative characters, by the absence of eyes and the lack of the brush-like chaetotaxy, of, for example, *Penicillidia*. The genus has been divided into three subgenera, *Nycteribia* (= *Listropoda* Kolenati), *Acrocholidia* and *Stylidia*. The subgenus *Nycteribia* is relatively well defined by the broad, short tibiae. The original definition of *Stylidia* is based mainly on the form of the anal lobes of the female which is apparently not a generic character, and the subgenus *Acrocholidia* contains some species which do not fit into the other two. A redefinition of the genus and the subgenera and a new distribution of species will become necessary after more species and particularly their genitalia have been re-examined. For the present the old divisions will be retained, but some new characters will be used for their definition and some species will be placed in different subgenera.

*Provisional diagnosis.* Small or medium-sized species with laterally compressed head. Eyes absent. Palps with two rows of setae ventrally and a very long apical seta. Labella of proboscis as long as theca or shorter. Notopleural sutures with broad lateral plates which bear a row of 6 to 15 notopleural hairs. Ctenidium of thorax with pointed spines. Tibiae flattened without marked rings and with several transverse rows of long setae distally. Haltere groove without cover. Male abdomen with normal segmentation. Tergites of female abdomen reduced to 4 or 5. Postspiracular sclerite narrow, strip-like, with several setae which are usually longer towards the free end, near the spiracle. Ctenidium of abdomen consisting of closely standing specialized spines. Male genitalia of *Nycteribia* type. Either two genital plates in the female, of which the dorsal one bears spines and the

ventral plate has a surface concave posteriorly, or only a dorsal plate which may or may not bear spines.

Subgenus *Nycteribia* subg. n.

Syn. *Listropoda* Kolenati, 1857.

Type species as for the genus.

Head partly membranous in anterior dorsal part. Tibiae short and broad, laterally compressed, with 3 rows of long setae on the ventral edge distally. Sclerotized plate lateral to notopleural sutures broad with a row of 8 to 15 hairs. Mesopleural suture wide and relatively short. In most species two genital plates present in the female, a dorsal bearing spines and a ventral without spines.

1. *Nycteribia pedicularia* Latreille, 1796

As pointed out in the section on nomenclature this was originally a composite species which was restricted by Kolenati's descriptions to its present definition.

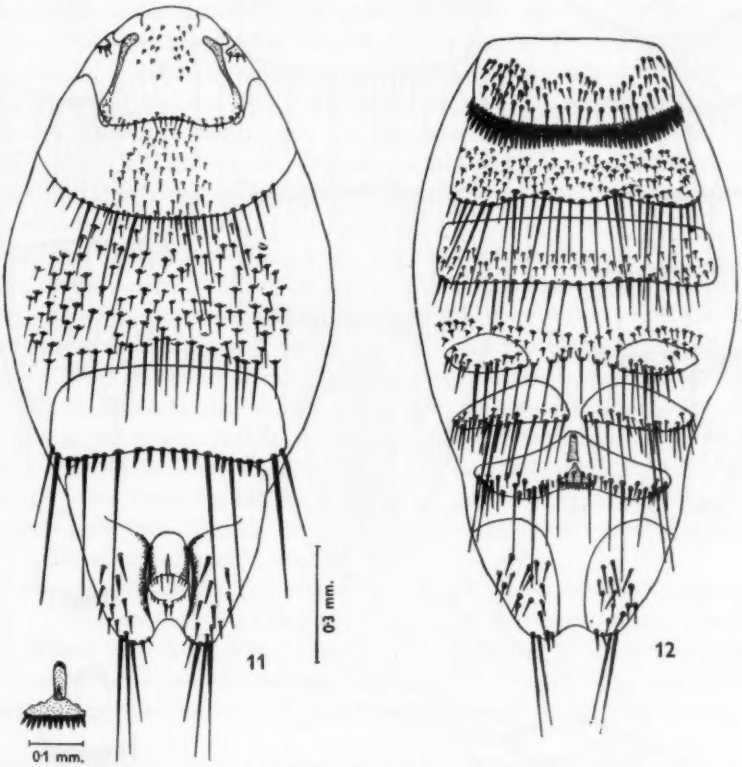
DESCRIPTION. Length 2·25–2·5 mm. Colour light yellowish brown.

*Head* moderately compressed laterally. Anterior dorsal part of the head membranous. Two to four short, widely spaced setae in the middle of the anterior margin of the head. A row of short setae along the anterior ventral margin of the head and a few setae on the genae. Labella of proboscis nearly as long as theca.

*Thorax* wider than long. Length 0·7 mm. Width 0·9 mm. Ctenidium with about twenty pointed teeth. Median sternal suture flattened except at the posterior end. Oblique sutures well marked, joining median suture at right angles. Mesonotal space moderately wide, slightly wider posteriorly. Lateral plate of notopleural suture wide with diagonal row of 6 to 9 hairs. Mesopleural suture wide and short, tapering laterally. Tibiae broad, short, compressed with curved ventral edge and three rows of setae in distal half, 2·25 times as long as broad.

*Abdomen female* (Figs. 11, 12). Tergite 1 roughly triangular with slightly concave posterior margin, some minute hairs along middle line and some short hairs along the posterior margin. Tergite 2 broad, slightly rounded posteriorly and with a row of 5 to 6 long and about 10 short setae among the long ones in the middle half of the hind margin. The tergite is covered with small hairs in the middle. Length of tergite 1 + 2 to width of tergite 2 = 3 : 4. (Tergites 1 and 2 have to be measured together as tergite 2 partly surrounds tergite 1 and the anterior limit of tergite 2 is difficult to determine.) Tergites 3–5 are fused into a membrane covered with about 6 to 8 rows of small spines which become gradually longer posteriorly. The posterior row consists of longer setae. The small hairs are restricted to the area between tergites 2 and 6 and the sides of the abdomen are bare. This is nearly constant in the Palestinian material where only a few specimens had a few scattered hairs near spiracles 4 and 5, while in a number of European specimens the sides of the abdomen were covered with hairs leaving only a narrow bare space between them and the hairs of the dorsal area. Tergite 6 narrow and bare, with six to eight long setae and about 15 short spines among them in groups of 1 to 6. Anal segment covered with short setae in its posterior lateral half and with several long setae at the tip of the short anal lobes. The area in front of the anus dorsally is covered by a rectangular sclerite.

Sternite 1 + 2 rectangular with a ctenidium of about 50 teeth and about 4 rows of short hairs in its posterior half. Postspiracular sclerite narrow and curved, with 1 or 2 longer setae near the spiracle, and 2 or 3 short setae towards sternite 1 + 2. Sternites 3 to 5 narrow, with a uniform row of long setae at the posterior margin and a few longer setae at the sides. Sternite 3 wholly covered with small hairs and sternite 4 with only 2 to 3 rows in the posterior half. Sternites 5 and 6 divided into two lateral plates. There are 4 to 5 setae between the plates of sternite 5,

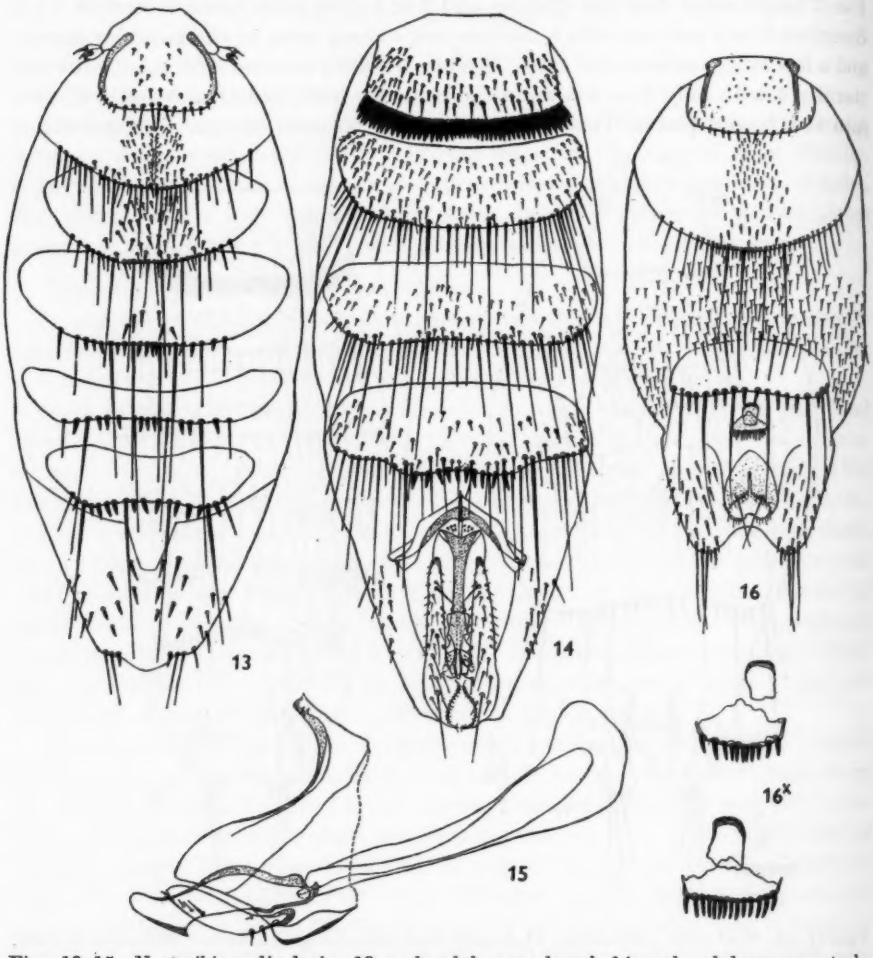



Figs. 11, 12. *Nycteribia pedicularia*. 11, female, abdomen, dorsal; 12, female, abdomen, ventral.

which bear a double row of long and short setae at the posterior margin. The plates of sternite 6 are larger and nearly reach the middle line. They bear similar setae to those of the plates of sternite 5. Sternite 7 triangular, concave posteriorly in middle with a double row of long and short setae at the sides and a single row in the middle. Dorsal genital plate triangular, with a row of about 12 short spines posteriorly. Ventral plate very narrow, with rounded anterior margin and concave posterior surface (Fig. 9*b*).

*Abdomen male* (Figs. 13, 14). Tergite 1 similar to that of female, but narrower. Tergite 2 also resembling that of female, but shorter. Tergite 3 similar but some setae on the posterior margin longer and hairs in the middle of the surface longer than on tergite 2. Tergites 4 to 6 bare with a row of long setae and short, strong

spines at the posterior margin. In some specimens there are a few hairs, rarely more than 6, in the middle of tergite 4. Anal segment conical, hairs as in female.




Figs. 13–15. *Nycteribia pedicularia*. 13, male, abdomen, dorsal; 14, male, abdomen, ventral; 15, male, genitalia, profile. Fig. 16. *N. latreillii*, female, abdomen, dorsal and genital plates.

Sternite 1 + 2 as in female. Sternites 3 to 5 longer than in female, covered with small hairs and with a row of long and short setae at the posterior margin. In the middle of the posterior margin of sternite 5 a row of 9 to 12 short spines (rarely 7 to 8, or 13 to 14). Twenty specimens from Palestine all had 9 to 12 spines. Among twenty-eight specimens from Europe, twenty-one had 9 to 12 spines, one had 7, one had 8, two had 13, and three had 14 spines. This group of spines is important as it gives a character for the separation of *N. pedicularia* from the related species *N. latreillii* and *N. kolenatii*.

*Genitalia* (Fig. 15). Basal arc broad and rounded with short anterior process.

Phallobase conical, with marked distal bulge. Dorsal hinge of phallobase pigmented, with two groups of small hairs between its base and the basal arc. Aedeagus rather short, with rounded tip and a subapical ventral tooth rather far posteriorly. Distance between tip and subapical tooth to length of aedeagus = 1 : 3. Apodeme moderately wide at the end. Parameres with blunt tip and 5 to 6 small hairs near the tip and 2 to 3 near the base. Claspers rather short and straight, tapering to a blunt dark tip, with a long seta near the base and some long hairs on the outside in the basal half and some small hairs near the tip.

Hosts. *N. pedicularia* was found in Palestine, commonly on the various species of *Myotis* and on *Miniopterus schreibersi*, and only a single specimen each on *Rousettus* and on *Tadarida teniotis*. None was found on the four species of *Rhinolophus*. Speiser and Falcoz record it from *R. hipposideros*, *Eptesicus serotinus*, *Nyctalus noctula*, *Myotis daubentoni*, *M. oxygnathus* and *M. dasycneme*. Some of these records are doubtful as *Nycteribia pedicularia* was not then distinguished from *N. latreillii* and *N. kolenatii*.

In addition to the Palestinian material, specimens from France, Italy, Germany, Austria, Bosnia and Turkey were examined. The Italian specimens were from *Myotis myotis*, *M. emarginatus* and *M. capaccinii*. The collection of the Museum in Vienna contained a great many specimens of this species, mostly determined as *Nycteribia blasii*. Some were found on *Myotis capaccinii* and some on *Rhinolophus* sp. in Dalmatia, Croatia and Bosnia. *Nycteribia pedicularia* has also been recorded from Britain by Smart (1939), but examination of his material in the British Museum from Henley on Thames (from *Myotis daubentoni* and *M. nattereri*) proved all to be *Nycteribia kolenatii*. Karaman gives a drawing of the aedeagus of some specimens from Tunisia found on *Myotis myotis* and mentions specimens from Yugoslavia also from *M. myotis*.

The records of *Nycteribia pedicularia* from South Africa refer to *N. capensis* Kar. and Scott's records from Formosa and China to *N. formosana* Kar. Scott records *N. pedicularia* also from Kashmir and from Darjeeling in India. The specimens from Kashmir have been re-examined and proved to belong to a new species. The eastern limit of distribution of *N. pedicularia* is thus not precisely known.

Seasonal distribution (Goldblum):

| | No. per bat | | | | |
|---|---|---|---|---|---|
| | May | June | July | Aug. | Sept. |
| *Miniopterus schreibersi* | 0 | 1–2 | — | 2–5 | 2–5 |
| *Myotis myotis* | 0 | 0 | 0–1 | 2–5 | 0–1 |
| *M. nattereri* | 0–1 | 2–5 | 2–5 | — | 1–2 |

2. *Nycteribia latreillii* Leach, 1817

Syn. *N. biscutata* Gil Collado, 1934.
*N. latreillii africana* Kar., 1939.

The original description of Leach and the descriptions of Curtis (1829) and Westwood (1835) are quite insufficient for the recognition of the species. They would fit *N. pedicularia* and *N. kolenatii* equally well. Leach gives the size of

*N. latreillii* as 1¼ lin. (approx. 2·7 mm.) and gives no locality. Curtis and Westwood record *N. latreillii* from Britain and Westwood gives the size as ¾ lin. (approx. 1·6 mm.). It seems, therefore, probable that Curtis and Westwood had before them *N. kolenatii*, the only species of the subgenus *Nycteribia* found in Britain to date. The difference in size given is much too large to lie within the range of variation of one species and as Leach gives no locality, but describes in the same paper another species from outside Britain, the possibility remains that his *N. latreillii* is the same insect which Kolenati described in detail in 1856 and 1863, which is of a size similar to that given by Leach. We therefore take as the basis for the definition of *N. latreillii* the description given by Kolenati.

DESCRIPTION. Length 2·8–3·25 mm. Colour light brown with darkly pigmented setae. Head similar to that of *N. pedicularia*.

*Thorax* (Fig. 3) similar to that of *N. pedicularia*. Length 0·9 mm. Width 1·1 mm. Median sternal suture narrow posteriorly, widening in middle, indistinct anteriorly. Sclerotized plate lateral to notopleural sutures more strongly marked than in *N. pedicularia* with a row of 9 to 12 hairs which begin a short distance behind the anterior spiracle. Mesopleural suture longer and narrower than in *N. pedicularia* and more darkly pigmented at the posterior margin. Legs as in *N. pedicularia*. Tibiae 2·35 times as long as broad.

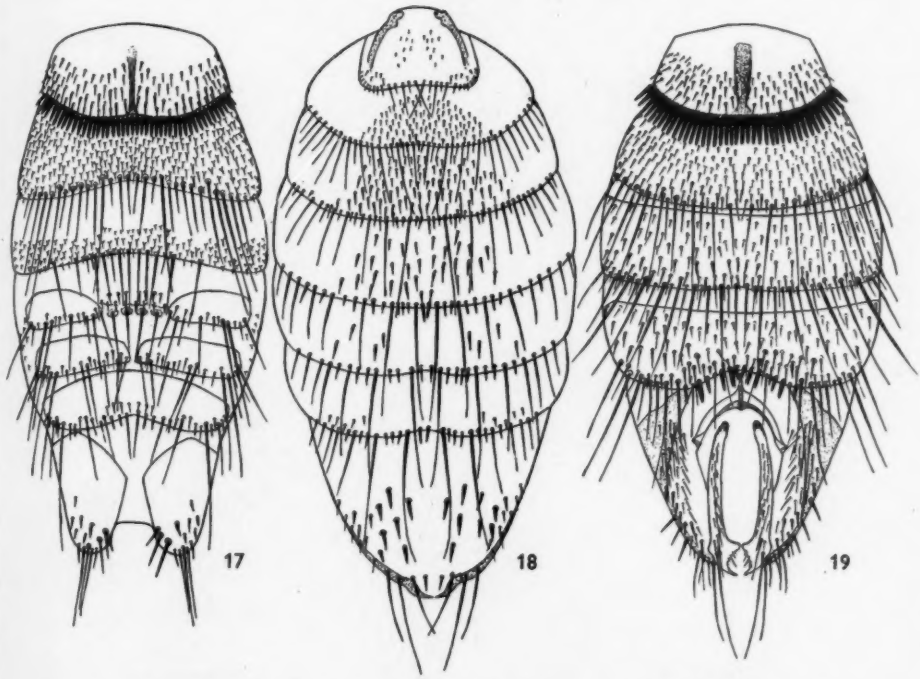
*Abdomen female* (Figs. 16, 17). Tergite 1 as in *N. pedicularia*. Tergite 2 longer and more strongly curved posteriorly than in *N. pedicularia* with 6 to 8 long setae in the middle of the posterior margin, groups of 1 to 4 short spines between the long setae and moderately long setae at the sides. All these setae are stronger and longer than in *N. pedicularia*. A large group of short hairs in the middle of the segment. Otherwise the dorsal side of the abdomen is much as in *N. pedicularia*, except that the sides of the abdomen down to the posterior level of tergite 6 are covered with small spines and are bare in *N. pedicularia*, and that all setae are darker and stronger. The sclerite in front of the anus dorsally is rounded or pointed anteriorly. The ventral side of the abdomen is also very similar to that of *N. pedicularia*. The dorsal genital plate (Figs. 9*a*, 16) is roughly triangular with truncated lateral corners and bears a row of 13 to 15 spines posteriorly which are longer and stronger than in *N. pedicularia*. Ventral plate broad, with rounded anterior margin and concave posterior surface. Postspiracular sclerite rather broad, slightly curved, with 3 to 4 longer setae near the spiracle and 1 or 2 short ones towards sternite 1 + 2. Sometimes all setae long.

*Abdomen male* (Figs. 18, 19). Similar to that of *N. pedicularia*, but there is a group of numerous small hairs covering the middle of tergite 4 and a few hairs in the lateral part of tergites 5 and 6. There are more and longer setae on the posterior margins of the tergites than in *N. pedicularia*. The group of spines in the middle of the posterior margin of sternite 5 consists of an irregular double row of 14 to 18 spines, most of which are longer than in *N. pedicularia* (Fig. 20).

*Genitalia* (Figs. 20, 21). Basal arc similar to that in *N. pedicularia*. Claspers longer than in *N. pedicularia*, with blunt, dark tips. Phallobase conical without the distal bulge present in *N. pedicularia*. Aedeagus tapering to a point with a ventral hook near the tip. The aedeagus is longer than half the apodeme. Para-

meres narrow with curved sides and blunt tip and a few hairs near the tip and base.

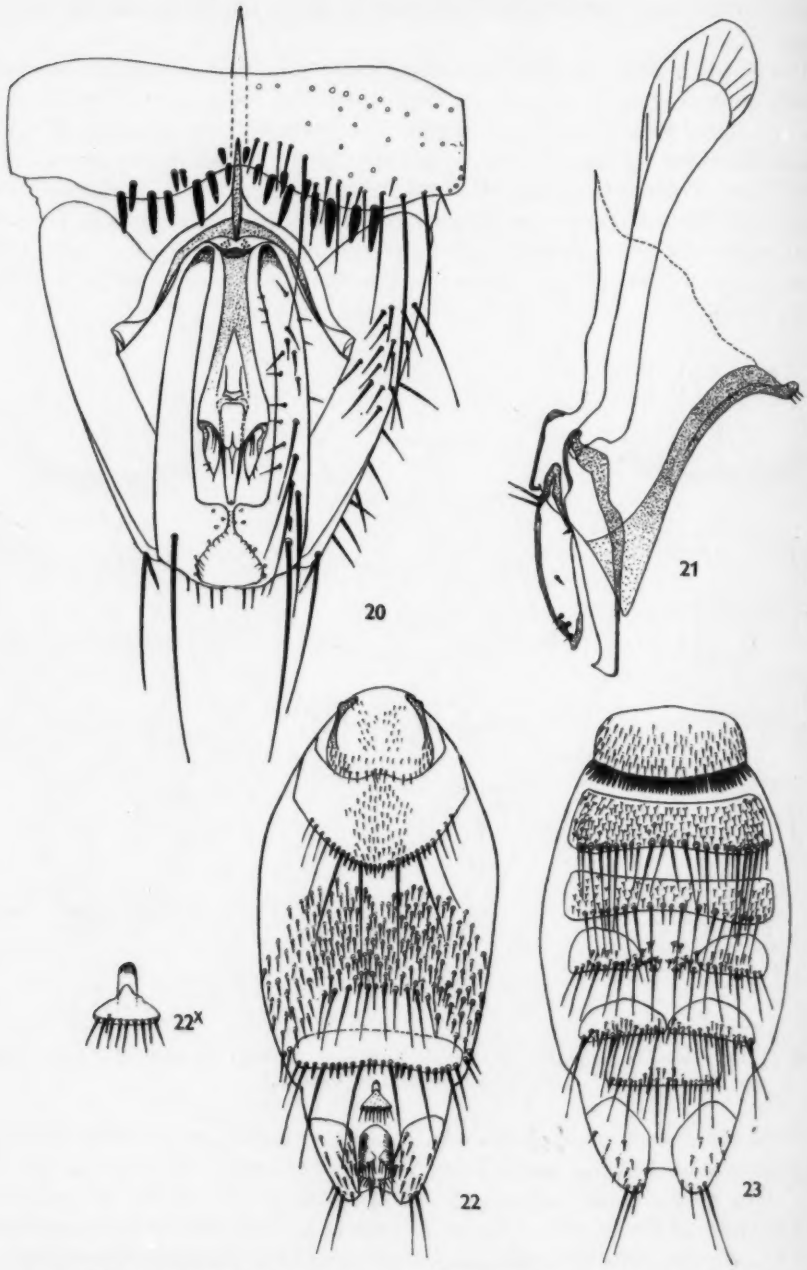
Hosts. *N. latreillii* was found in Palestine only on *Myotis myotis* in northern Galilee from August to October (twenty specimens). Karaman records it from Sofia on *M. myotis* and also from Hungary, Moravia and Yugoslavia from *M. myotis* and *Rhinolophus ferrumequinum*. In addition to the Palestinian material, specimens from France, Germany, Holland, Yugoslavia, Italy and Morocco were examined. *Plecotus auritus* or *Rhinolophus hipposideros* were given as hosts for some specimens from Germany and *Vespertilio murinus* (= *Myotis myotis* ?) for some specimens from France. Most specimens from Italy were found on *M. myotis* and a few on *M. capaccinii* and on *Rhinolophus euryale*.




Figs. 17–19. *Nycteribia latreillii*. 17, female, abdomen, ventral; 18, male, abdomen, dorsal; 19, male, abdomen, ventral.

Karaman records a subspecies *africana* from Tunisia, which differs from the typical form only in size and in minor details of the form of the parameres. The size given by Karaman, however, is within the range of variation of the species and the form of the tip of the paramere varies to a certain extent. Two males from Morocco did not differ from European material and the retention of the subspecies therefore does not seem justified.

The specimen recorded by Corradetti (1934) from the Roman Campagna as similar to *Nycteribia blasii* is also *N. latreillii*.




Figs. 20, 21. *Nycteribia latreillii*. 20, male, sternite 5 and genital area; 21, male genitalia, profile. Figs. 22, 23. *N. kolenatii*. 22, female, abdomen, dorsal and genital plates; 23, female, abdomen, ventral.

Gil Collado (1934) described a new species, *N. biscutata*, from near Valencia in Spain, found on *Eptesicus serotinus*. Through the kindness of Prof. Ceballos of the Museum in Madrid, I had the opportunity to examine the specimen. It proved to be an abnormal specimen (♀) of *Nycteribia latreillii* in which tergite 6 is divided into halves, each of which bears slightly more than its usual number of setae. In all other respects, e.g. genital plates, the specimen is a typical *N. latreillii*. The name *N. biscutata* thus becomes a synonym of *N. latreillii*.

3. *Nycteribia kolenatii* n.nom.

Syn. *N. blasii* Kolenati, 1863 (nec 1856).
? *N. latreillii* Curtis, 1829.
? *N. latreillii* Westwood, 1835.
nec *N. latreillii* Leach, 1817.
nec *N. latreillii* Kolenati, 1856 and 1863.

Although this species is not represented in the collection from Palestine, the discussion of its nomenclature and a description is given in order to clarify its relationship to the other species of the subgenus.

The original description of *N. blasii* given by Kolenati in 1856 doubtless refers to *N. schmidlii* Schiner, 1853. Kolenati states that the 4th ventral segment (=sternite 5) of the male is 'kahl, glänzend, mit *stark bogigem*, nach abwärts gerichteten Hinterrande, welcher in der Mitte ein 14 zähniges Querctenidium.. trägt'. He also speaks of 5 tergites in the female and describes the characteristic 3rd tergite as having 8 upward-pointing, short setae and 3 long setae on each side. The writer has a copy of Kolenati's paper of 1856 which bears a dedication in his own handwriting. Under the name *N. blasii* the remark is written, also in his own characteristic handwriting: '*schmidlii* Schiner, Fauna der Adelsberger Grotte und Verh. Zool. Bot. Vereines 1853'. In his paper of 1863, however, he gives clear figures and a correct description of *N. schmidlii* under this name and gives a new description of another species to which he now gives the name *N. blasii*. Furthermore, Kolenati gives as host for *N. blasii*, 1856: '*M. schreibersi*, sehr häufig, Banat, Serbien, Dalmatien'. In his paper of 1863 he gives the same host and the distribution: 'Serbia, Banat, Krain, Roman Campagna for *N. schmidlii*, and as hosts for *N. blasii*: *P. nathusii*, *M. daubentoni*, and *M. capaccinii*, with the distribution: Austria, Hungary, Russia, Silesia, Prussia'. This corresponds to our present knowledge. *N. schmidlii* is mainly a parasite of *Miniopterus schreibersi*, while none of the specimens of the species called *Nycteribia blasii* by Kolenati in 1863 were found on this bat, but on species of the genus *Myotis* (*M. daubentoni*, *M. mystacinus*, *M. nattereri*), on *Eptesicus serotinus* and others.

The name *Nycteribia blasii* Kolenati, 1856 must therefore be considered as a synonym of *N. schmidlii* Schiner, 1853, and we propose the name *N. kolenatii* n.nom. for the species called *N. blasii* by Kolenati in 1863 (nec 1856).

*N. kolenatii* is probably the insect called *N. latreillii* by Curtis (1829) and Westwood (1835) as pointed out above in connexion with the nomenclature of *N. latreillii*.

Description. Length 2–2·25 mm. Colour yellowish. Head, thorax and legs similar to those of *N. pedicularia*, except that the tibiae are slightly shorter.

*Abdomen female* (Figs. 22, 23). In general similar to *N. pedicularia*, but tergite 2 is longer and more strongly convex or even triangularly produced posteriorly

with about 4 long setae in the middle of the posterior margin and groups of 2 to 6 short spines among the long ones and a few shorter hairs at the sides. This arrangement resembles more closely that in *N. latreillii* than that in *N. pedicularia*. Length of tergites 1 and 2 nearly equal the width of tergite 2. The small hairs on the sides of the abdomen extend posteriorly to the level of tergite 6, but they are not as numerous and do not extend as far as in *N. latreillii*. The sclerite in front of the anus dorsally is rounded anteriorly. Sternite 7 is nearly straight anteriorly, not triangularly produced as in *N. pedicularia*. Dorsal genital plate triangular with 8 to 10 setae which are about as long as the plate itself and stand more widely spaced than the short spines in *N. pedicularia* and *N. latreillii*. Ventral genital plate narrow, similar to that in *N. pedicularia*. Postspiracular sclerite rather short and triangular, with one or two longer setae near the spiracle and 3 to 4 shorter setae near sternite 1 + 2.

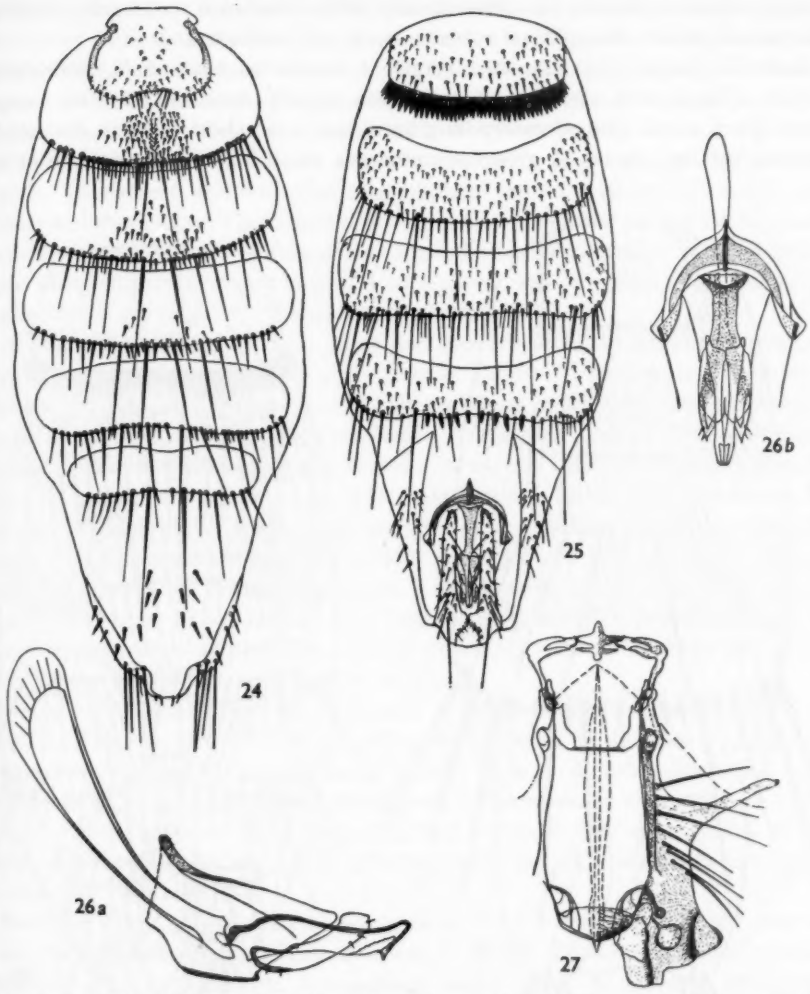
*Abdomen male* (Figs. 24, 25). In general similar to that in *N. pedicularia*, but there are only rarely a few small hairs on tergite 4. The row of short spines in the middle of the posterior margin of sternite 5 consists of 7 to 8 spines as a rule. Among twenty specimens examined, one had 5, fourteen had 7 to 8, three had 9 and one had 10 spines.

*Genitalia* (Fig. 26*a*, *b*). Claspers rather short and tapering slightly to a blunt point. Basal arc widely rounded. Phallobase conical without the distal bulge characteristic for *N. pedicularia*. Aedeagus relatively longer and slenderer than in *N. pedicularia*, more than half the length of the apodeme. Subapical tooth of aedeagus closer to the tip than in *N. pedicularia*. Distance from tip to subapical tooth to length of aedeagus = 1 : 4·5 or 1 : 5. Parameres with blunt tip and 3 short hairs near tip.

MATERIAL EXAMINED. Three type specimens of Loew's collection (Zool. Museum, Berlin) from *Myotis daubentoni*. Four specimens from Kolenati's collection in the Museum in Paris. Six specimens from *M. daubentoni* and *Eptesicus serotinus*, Anger, France. Three specimens from *P. pipistrellus* and *Myotis mystacinus*, Rouen. Six specimens from Henley on Thames from *M. daubentoni* and *M. nattereri*, identified as *Nycteribia pedicularia* (Coll. British Museum). Several specimens from Austria (Tyrol) and Germany without indication of host and some specimens from Holland from *Myotis daubentoni*, *M. dasycneme*, *M. mystacinus* and *Vespertilio murinus* (= *Myotis myotis* ?) from January to August (Museum Amsterdam). Some specimens from *M. daubentoni*, Pisa and Tosrana in Italy were also examined.

*Nycteribia kolenatii* is thus known with certainty only from Britain, France, Italy, Holland, Austria and Germany. Records from other localities may refer to *N. pedicularia* or other species, as even specimens identified by Speiser himself as *N. blasii* were found to be either *N. schmidlii* or *N. pedicularia* (Vienna Museum).

The distribution of *N. kolenatii* thus coincides with that of *N. pedicularia*, but is more restricted. Karaman considered *N. blasii* as a subspecies of *N. pedicularia*. But although both forms are closely related, the differences in the shape of tergite 2 of the female, the dorsal genital plate, the structure of the male genitalia and the constant difference in the number of spines on sternite 5 of the male justify the retention of *N. kolenatii* as a separate species.




Figs. 24–26. *Nycteribia kolenatii*. 24, male, abdomen, dorsal; 25, male, abdomen, ventral; 26, male genitalia: *a*, profile; *b*, ventral. Fig. 27. *N. schmidlii*. Dorsal pattern of thorax.

4. *Nycteribia schmidlii* Schiner, 1853

Syn. *N. blasii* Kolenati, 1856 (nec 1863).

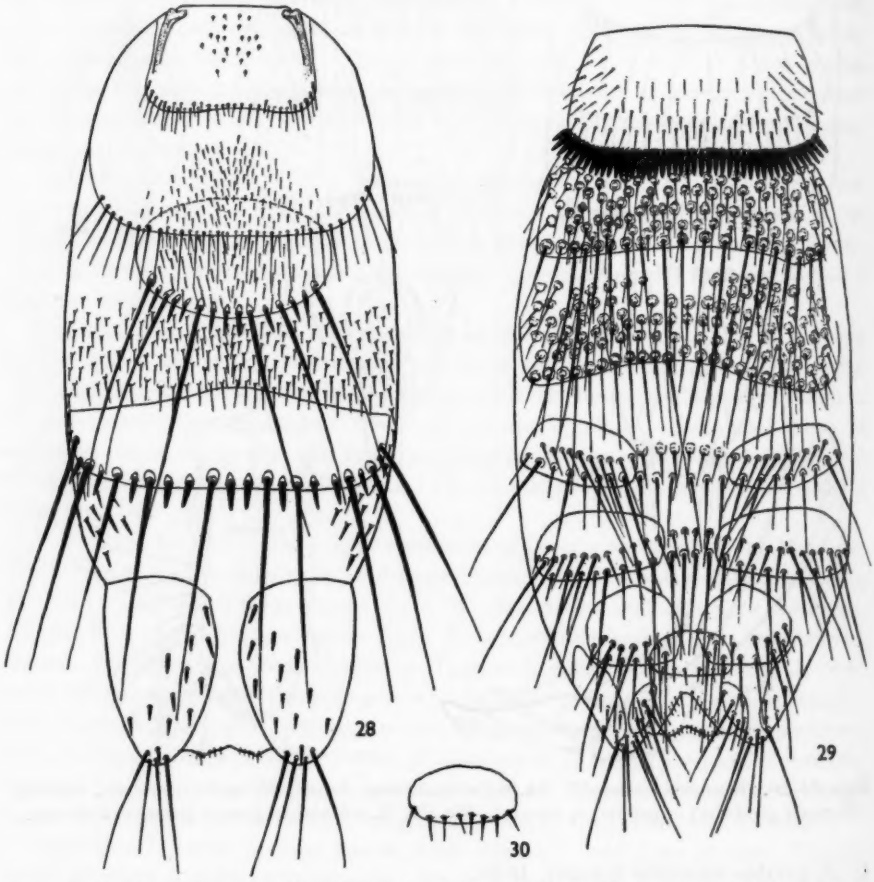
Description. Length 2–2·25 mm. Colour light yellowish brown.

*Head* similar to the preceding species. Setae on front margin longer and standing more closely together than in the other species of the subgenus.

*Thorax* (Fig. 27) about as long as wide. Length 0·75 mm. Width 0·8 mm. Median ventral suture well marked, less flattened in middle than in *N. pedicularia*. Angle between oblique sutures slightly less than 90°. Dorsal pattern: notopleural sutures nearly parallel, relatively close together. Lateral plates of notopleural

sutures narrower than in the other species with a row of 6 to 9 hairs. Anterior spiracles elliptical. Mesopleural suture narrow and well marked.

*Abdomen female* (Figs. 28, 29). Tergite 1 similar to that of *N. pedicularia*. Tergite 2 large with straight or sometimes slightly concave posterior margin which bears a row of moderately long hairs and some short hairs in the middle between the long hairs. A group of numerous small hairs in the middle of the




Figs. 28–30. *Nycteribia schmidlii*. 28, female, abdomen, dorsal; 29, female, abdomen, ventral; 30, female, dorsal genital plate.

tergite. Tergite 3 well defined, narrower than the second, elliptical, with a row of 5 to 7 very long setae and 8 to 10 short spines between the longer setae. Its surface covered with small hairs. Tergites 4 and 5 fused into a membrane which is covered with small hairs between tergites 3 and 6, leaving the sides of the abdomen bare. Tergite 6 bare, narrow, with 8 to 10 long setae and 10 to 12 short spines between them. Some small spines on the membrane of the abdomen laterally and posteriorly

to the tergite. Anal segment divided into two lobes which bear small spines on their inner surfaces and about 4 long setae at the tip. There is no sclerite dorsally in front of the anus. Sternite 1 + 2 similar to that of the other species of the subgenus with a ctenidium of 40 to 45 teeth. Postspiracular sclerite similar to that of *N. pedicularia*, but shorter and less curved. Sternites 3 and 4 covered with short hairs on chitinous tubercles and a row of uniformly long setae at the posterior margin. Sternites 5 and 6 divided into elliptical lateral plates which bear 2 rows of long and short setae. These rows continue in the space between the lateral plates. Lateral plates of sternite 7 irregularly rounded and close together. Dorsal genital plate wide, elliptical, with a row of 4 to 8 short, widely spaced hairs before the posterior margin (Fig. 30). Ventral plate absent.

*Abdomen male* (Figs. 31, 32). Long and slender and held curved downwards. Tergites 1 and 2 similar to those of the female. Tergite 3 with a group of numerous small hairs in the middle and a row of long and short setae at the posterior margin. Tergite 4 more strongly convex than tergite 3 and with a group of larger hairs in the middle. Posterior row of setae also similar to that of tergite 3 except that the setae are longer. Particularly two in the middle are very long and there are a few short spines near them. Tergite 5 strongly convex posteriorly, bare, with a similar row of setae, of which 4 near the middle are especially long. Tergite 6 narrow, strip-like, bare, often covered by tergite 5 and bearing a row of moderately long setae posteriorly. Anal segment very long, as long as the 4 preceding segments together, and with nearly parallel sides. A group of short spines in the middle and a few longer ones at the sides and at the tip.

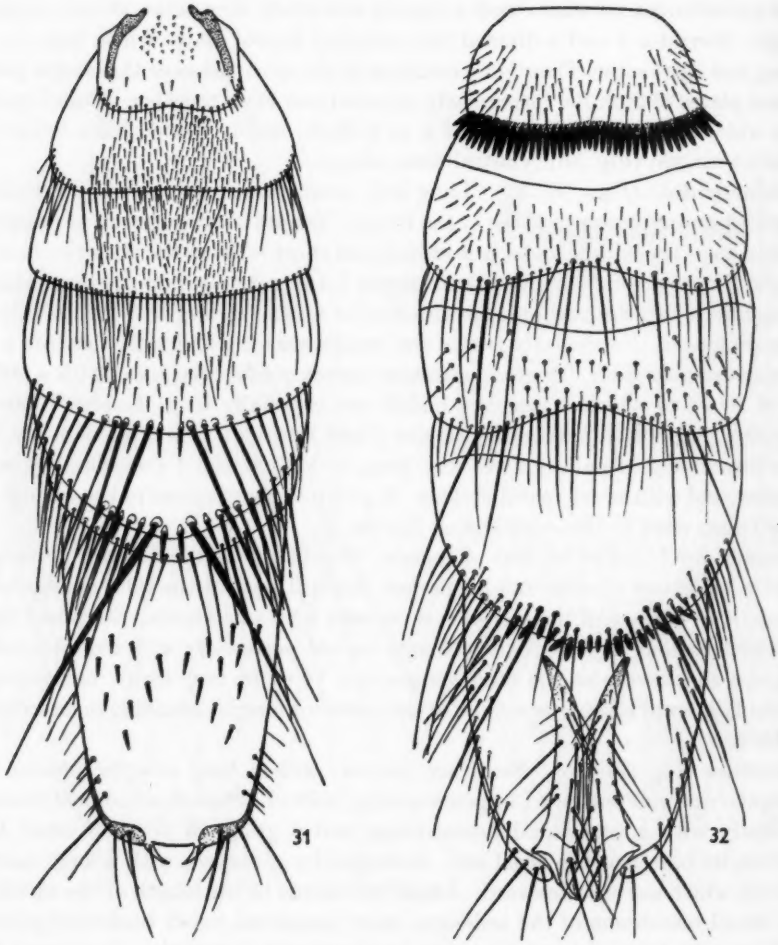
Sternite 1 + 2 similar to that of female. Sternites 3 and 4 concave posteriorly with the usual row of short and long setae. Several setae at the sides are especially long and the surface of the sternites is covered with small hairs. Sternite 5 large, two-thirds as long as wide, bare, strongly curved posteriorly with a double row of long and short setae at the hind margin and 14 to 16 very short, barrel-shaped spines of different size in the middle of the posterior margin, standing in an irregular double row.

*Genitalia* (Fig. 33*a*, *b*). Basal arc narrow, with a long anterior process and triangular anterior outline. Claspers tapering, curved. Phallobase conical, rounded anteriorly with a pigmented dorsal hinge and a group of about 8 small hairs between its base and the basal arc. Aedeagus long, curved, with a long, narrow, bifid tip which curves backwards, about two-thirds of the length of the apodeme. The dorsal membrane of the aedeagus bears numerous small backward-pointing teeth near its base and terminates in two processes anteriorly. Parameres very narrow, slightly curved, parallel-sided with rounded tip and 3 short hairs near it and two small hairs at the lower edge.

*N. schmidlii* belongs to the subgenus *Nycteribia* according to the structure of head and tibiae, but differs markedly from the three preceding species in the form of the abdominal sclerites, chaetotaxy, the structure of the male genitalia and the absence of a ventral genital plate in the female.

Hosts. *N. schmidlii* was found in Palestine mainly on *Miniopterus schreibersi*. Only a few specimens were found on *Myotis myotis* and a single specimen each on

*Asellia tridens* and on *Rousettus aegyptiacus*. Karaman also records it mainly for *Miniopterus schreibersi* and rarely for various species of *Rhinolophus*. Falcoz gives as hosts, in addition to *Miniopterus schreibersi* and *Myotis myotis*, *Rhinolophus mehelyi*, *Myotis daubentoni* and *P. pipistrellus*.

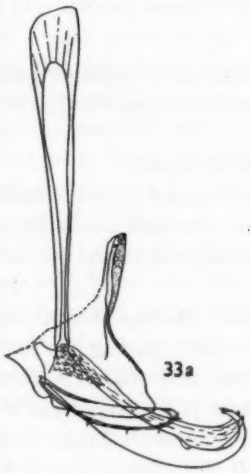


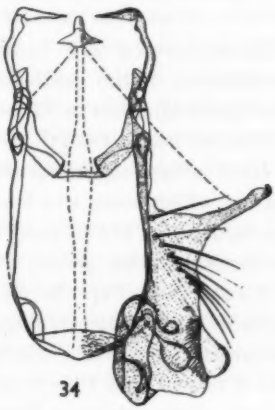

Figs. 31, 32. *Nycteribia schmidlii*. 31, male abdomen dorsal; 32, male, abdomen, ventral.

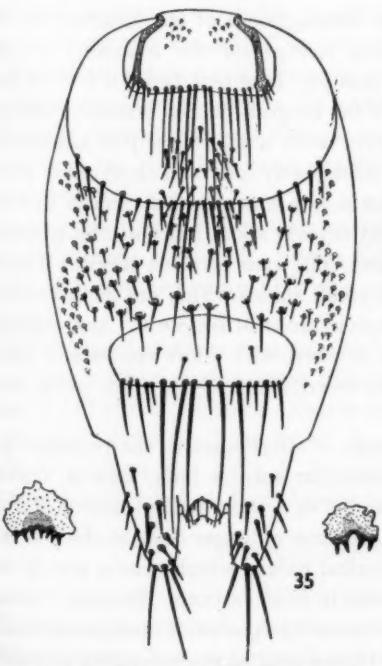
In addition to the Palestinian material, specimens from the following countries and hosts were examined: Italy, France, Spain and Dalmatia from *Miniopterus schreibersi*. A few specimens from Italy were found on *Rhinolophus* sp., *Myotis myotis*, *M. daubentoni* and *M. capaccinii*. France on *M. myotis* and *Rhinolophus ferrumequinum*. Bosnia and Croatia on *Rhinolophus* sp. Algeria on *R. ferrumequinum*. Morocco, Antioch, Turkey on *R. ferrumequinum*.

SEASONAL DISTRIBUTION (Goldblum).

| | No. per bat | | | |
|---|---|---|---|---|
| | June | July | Aug. | Sept. |
| *Miniopterus schreibersi* | 0–1 | — | 5–10 | 2–5 |
| *Myotis myotis* | 0 | 0 | 0–1 | 0–1 |





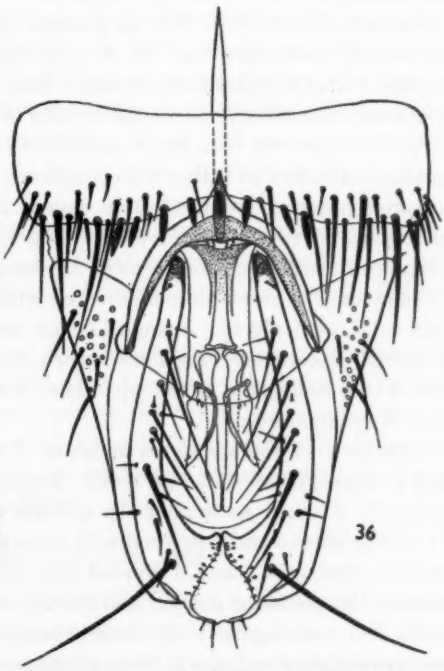




Fig. 33. *Nycteribia schmidlii*. Male genitalia: *a*, profile; *b*, ventral. Figs. 34–36. *N. vexata*, 34, dorsal pattern of thorax; 35, female, abdomen, dorsal and dorsal genital plate; 36, male, sternite 5 and genital area.

Specimens from tropical and South Africa show some constant minor differences in chaetotaxy and may eventually have to be considered as a separate subspecies.

Subgenus *Acrocholidia* Kolenati, 1857

5. *Nycteribia* (*Acrocholidia*) *vexata* Westwood, 1835

Syn. *N. bechsteini* Kol. 1857.
*N. ercolanii* Rond. 1879.

The synonymy of *N. vexata* and *N. ercolanii* was established by examination and preparation of the genitalia of some specimens of Rondani's type series in the Zoological Museum in Florence.

DESCRIPTION. Length 2–2·5 mm. Colour light yellowish brown.

*Head* rather long, moderately compressed laterally, sclerotized up to the anterior margin which bears 4 to 6 setae in the middle, the lateral of which are longer than the median. A row of short hairs along the anterior ventral margin and one or two short setae on the genae. Eyes absent (Fig. 1*b*).

*Thorax* (Fig. 34). As wide as long. Length 0·85 mm. Median sternal suture flattened in middle. Oblique sutures well marked and wide, joining the median suture at an angle of about 80°. Dorsal pattern rather similar to that of *N. schmidlii*. A row of 8 to 10 notopleural hairs begins rather more posteriorly than in the species of the subgenus *Nycteribia*. Ctenidium with about 20 teeth. Tibiae slender, scalpel-shaped, with 3 rows of long setae in the distal half, 3·5 times as long as wide.

*Abdomen female* (Fig. 35). In general the arrangement of the tergites and the chaetotaxy resembles that of *N. pedicularia* except for the following details: tergites 1 and 2 with nearly straight hind margin. Tergite 1 bears a row of long thin setae, of which those in the middle are the longest, on the posterior margin. Tergite 2 is shorter than in *N. pedicularia* and bears a small group of hairs in the middle, and a row of rather widely spaced, moderately long and short setae at the posterior margin. Tergite 6 bare except for a few hairs in the middle in some specimens. A row of about 4 or 5 long and several short hairs at the posterior margin, of which the median setae are longest. The area between tergites 2 and 6 and the sides of the abdomen covered with small spines which are less numerous than in *N. pedicularia*. A row of longer setae in front of tergite 6. Anal segment produced into rather long lobes which are covered with short spines and which bear 3 to 4 long setae at the tip. There is no sclerite dorsally in front of the anus as in *N. pedicularia*.

Sternite 1 + 2 rectangular with about 3 rows of short hairs in the posterior half and a ctenidium of 45 to 50 teeth. Postspiracular sclerite long, narrow, curved, with 2 to 3 longer setae near the spiracle and 3 to 4 short setae towards sternite 1 + 2. Sternites 3 and 4 covered with hairs and a row of longer hairs at the posterior margin. Sternites 5 and 6 divided into elliptical halves which bear a row of long setae at the posterior margin and shorter setae in front of them. Sternite 7 broad, undivided, rectangular with a row of long setae at the posterior margin and one or two rows of shorter hairs in front of them. Dorsal genital plate roughly triangular or rounded anteriorly, with a concave, more heavily sclerotized posterior margin which bears 4 to 6 widely spaced short spines. Ventral plate absent.

*Abdomen male.* Tergite 1 similar to that of female, but smaller and the hairs on the hind margin are shorter. Tergites 2 to 6 rather narrow with uniform rows of hairs posteriorly, which are longer on the posterior tergites, particularly 4 setae in the middle of tergite 6. There are groups of small hairs in the middle of tergites 2 to 4, tergites 5 and 6 are bare. Anal segment rather short, narrow and conical with two rows of moderately long hairs in the posterior half and at the sides, and a few longer setae at the tip. Sternite 1+2 as in female, but more rounded anteriorly. Sternites 3 and 4 narrow, with uniform rows of moderately long hairs posteriorly, and 2 to 3 rows of short hairs on the surface. Sternite 5 slightly concave posteriorly, with a double row of long setae and generally a single row of 7 to 8 short spines in the middle of the posterior margin. Of twenty-one specimens examined, one had 5, three had 6, fourteen had 7 to 8, two had 9, and one had 10 spines (Fig. 36).

*Genitalia* (Figs. 36, 37). Basal arc broad and rounded. Claspers thin, slightly curved with blunt tips, a long seta near the base and several long and short setae nearly up to the tip. Phallobase conical with triangular narrow dorsal hinge and a few small hairs between it and the basal arc. Aedeagus broad, thinly chitinized, with broadly rounded tip, longer than half the apodeme. Parameres narrowly triangular, with blunt tip which bears 3 small hairs. Two more small hairs near the base.

Hosts. *N. vexata* was found in Palestine only on *Myotis myotis* in Northern Galilee from June to October, together with *Nycteribia latreillii*. About twenty specimens were examined. Karaman also records *Myotis myotis* as principal host of *Nycteribia vexata* in Yugoslavia and Tunisia, and in addition *Rhinolophus ferrumequinum* and *R. euryale*. In addition to the above species Speiser and Falcoz mention *R. hipposideros* and *Miniopterus schreibersi*. Scott (1925) records *Nycteribia vexata* from the Himalayas and mentions a record from Algeria from *Myotis blythi oxygnathus*.

In addition to the Palestinian material, specimens from the following localities were examined: Persia, Mt. Elburz (*M. myotis*) (Coll. Brit. Museum), Constantinople, Morocco, Germany (*Plecotus auritus*, *Rhinolophus hipposideros*), France, Bosnia.

*Nycteribia bechsteini* was separated from *N. vexata* by Kolenati, as Speiser puts it, on the basis of such minute differences, that these may well fall within the range of individual variation. This is borne out by the figures given above for the spines on sternite 5 of the male. Kolenati gives 10 such spines for *N. vexata* (=*N. montaguei*) and 5 for *N. bechsteini*. A number of specimens of *N. vexata* examined were labelled *N. bechsteini* by Falcoz.

### Subgenus *Stylidia* Westwood, 1840

Syn. *Celeripes* Montagu, 1808.

Type species: *Nycteribia biarticulata* Hermann, 1804.

The subgenus is based mainly on the presence of the long processes on the abdomen of the female of *N. biarticulata*. This character in itself is not sufficient for the definition of the subgenus. Many species of Nycteribiidae have the anal

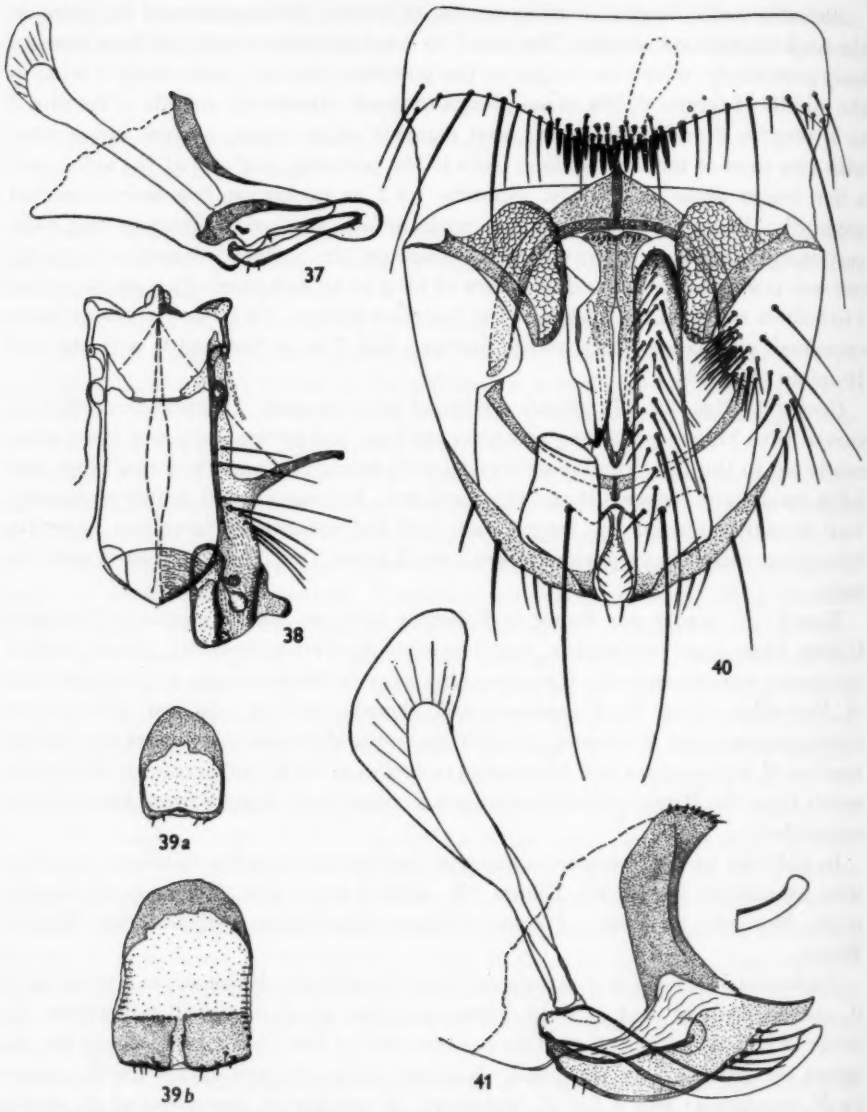



Fig. 37. *Nycteribia vexata*. Male, genitalia, profile. Fig. 38. *N. biarticulata*. Dorsal pattern of thorax. Fig. 39. Dorsal genital plates of female of: *a*, *N. biarticulata*; *b*, *N. biloba*. Figs. 40, 41. *N. biarticulata*. 40, male, genital area and sternite 5; 41, male genitalia, profile.

lobes prolonged to a varying degree, and a specimen of a new species of *Basilia* from Italy examined recently has long processes on the abdomen similar to those of *Nycteribia biarticulata*.

Although according to the old classification *N. biloba* n.sp. and *N. integra* n.nom. should be placed into the subgenus *Acrocholidia*, they are much more closely

related to *Nycteribia biarticulata* than to any species of the subgenus *Acrocholidia*, and they are therefore provisionally placed into the subgenus *Stylidia*, although they lack the long abdominal processes of *Nycteribia biarticulata*. A new diagnosis of the subgenus will have to be based mainly on the structure of the male and female genitalia and the characteristic segmentation of the female abdomen.

PROVISIONAL DIAGNOSIS. This is based on six species studied and will probably have to be modified when more species will have been examined.

Head sclerotized nearly up to front margin. Median sternal suture narrow, not much widened in middle. Lateral plate of notopleural suture present only in posterior half. Notopleural hairs more widely spaced anteriorly than posteriorly. Tibiae, long, scalpel shaped, 4 to 5 times as long as broad, with a group of small hairs near the basis of tibia 1 on the anterior surface. Row of setae of ventral hind margin of thorax reduced, with a wide gap in the middle. Post-spiracular sclerite rather short and straight with several long setae. Second tergal plate of female very wide. Anal processes of female very long or absent. Only a dorsal genital plate with a few minute spines present. Sternite 5 of male with several rows of spines near hind margin which may be divided into two lobes in some species. Phallobase strongly concave dorsally. Aedeagus curved. Parameres fused partially or completely with phallobase and with pointed process which has a number of hairs near its base.

6. *Nycteribia* (*Stylidia*) *biarticulata* Herm., 1804

DESCRIPTION. Length 2·5–3 mm. Colour light brown.

*Head* moderately compressed anteriorly, sclerotized nearly up to the anterior margin which bears 4 to 6 moderately long setae in the middle. A row of short hairs along the anterior part of the ventral margin of the head and one longer and one shorter seta in the posterior part. A few hairs on the genae. Eyes absent.

*Thorax* (Fig. 38) as long as wide. Length 1·1 mm. Median sternal suture well marked, slightly widened in the middle. Oblique sutures well marked, joining median suture at an angle of 75°. Dorsal pattern: mesonotal space narrow, parallel sided. Anterior spiracles round, situated at the posterior limit of the insertion of the head. Lateral plates of notopleural sutures present only in posterior half of suture, rather narrow, with a row of 10 to 13 hairs which begins some distance behind the spiracles. Spaces between anterior hairs much wider than between posterior hairs. This is quite constant in the Palestinian material, but not so marked in the European material. Mesopleural suture well marked, narrow and long, originating rather far posteriorly. Ctenidium with 18 to 20 long pointed teeth. Tibiae long and narrow, scalpel shaped, laterally compressed, about 4·5 or 5 times as long as wide (Fig. 4*a*). In the basal half of the anterior surface of the fore tibia a group of small spines which Karaman considers as characteristic for this species, but which is also present in *N. biloba*, *N. integra*, the South African *N. scissa* and *N. hindlei*. There is a similar smaller group near the apical end of the hind tibia. Three transverse rows of setae in the apical half of the tibiae.

*Abdomen female*. Tergite 1 broader than long, bilobed posteriorly. A row of moderately long hairs in the middle of the hind margin of each lobe, leaving the

indentation between the lobes bare. A few small hairs on the surface of the tergite. Second tergal plate, which probably consists of the fused tergites 2 to 4, large, rounded and incompletely divided by a median line. Posterior margin with a row of 12 to 16 long setae at the sides and only short setae in the middle. Some small spines along the median line at each side. Third tergal plate, which probably consists of the fused tergites 5 and 6, large, roughly triangular or rounded behind, with 3 to 4 long and short setae in the middle of the hind margin and a few scattered short setae on the surface. The 2 lateral setae are much shorter than the median setae in the Palestinian specimens. The anal tergite surrounds the segment completely. It is broad and rounded and bears dorsally two long thin processes which bear 4 long setae at the tip, forming a cross at right angles to the process, and a few short setae at the stem. There are two short setae near the anus at the ventral junction of the two sides of the tergite.

Sternite 1 + 2 narrower than the abdomen, rather long. The ctenidium consists of about 45 teeth. There are 3 irregular rows of short hairs in the posterior half of the sternite. Postspiracular sclerite short and straight with 3 to 4 long setae near the spiracle and several shorter setae towards sternite 1 + 2. Sternite 3 with 4 rows of strong setae which are short anteriorly and moderately long posteriorly. Sternite 4 with uniform row of moderately long setae posteriorly and a row of short setae before them. Sternites 5 and 6 narrow, divided into lateral plates with straight or concave hind margins which bear a row of longer setae at the posterior margin and a row of shorter setae on the surface. The plates of both segments reach the middle line. Some setae at the posterior lateral angles are longer than the others. Sternite 7 as long as sternites 5 and 6 together, divided into two roughly triangular plates, each of which bears two rows of setae, the posterior row consisting of longer setae with the longest at the lateral angles. Sides of the abdomen covered with short spines standing on tubercles. Only a dorsal genital plate present. It is shield-shaped with more strongly chitinized anterior margin and an indentation in the posterior margin which bears 4 small hairs (Fig. 39*a*). It is 0·18 × 0·13 mm. in Palestinian specimens and 0·22 × 0·18 mm. in European specimens.

*Abdomen male.* Tergite 1 similar to that of female, tergites 2 to 6 narrow with a row of moderately long and short setae posteriorly and 2 to 4 very long setae in the middle of the posterior margin of tergites 5 and 6. Tergites 2 to 5 with groups of small hairs in the middle, tergite 6 bare. Anal segment wide and rounded with two transverse rows of short hairs in the middle. Ventral side of abdomen similar to that of female, but sternite 5 longer, convex in middle posteriorly, where there is a group of 4 to 5 rows of short strong spines. Those of the posterior row are about 4 times as long as the spines of the anterior row and the spines between them are of intermediate length. The surface of the sternite is bare except for a row of short setae in front of the group of spines. The posterior border bears 2 to 4 very long and a few shorter setae. At the ventral surface of the last abdominal segment, at its anterior lateral angle, there are two thick, rounded processes with a more strongly sclerotized band posteriorly. These processes, which bear no hairs and have a reticulated surface, can be erected. They are probably used during copu-

lation to hold the female between them and the claspers. Posteriorly to these processes, there are two bulges, thickly covered with hairs, and some setae lateral to these bulges (Fig. 40).

*Genitalia* (Figs. 40, 41). Basal arc wide and rounded, darkly pigmented in some specimens and with a short anterior process. Claspers thick and straight, with blunt, dark tips and a double row of hairs along their outer surface. There is a long seta near the base and the hairs become gradually shorter towards the tip. Phallobase short, strongly arched, with broad pigmented dorsal hinge which bears 10 to 12 short hairs near the base. Aedeagus narrow, curved and tapering to a knoblike tip. Apodeme relatively short with broad apical plate. Aedeagus about two-thirds of length of apodeme. Parameres broad, partly fused with the phallobase, with a beak-like distal process which bears 5 to 6 hairs at its base in the middle of the lower edge.

Hosts. *N. biarticulata* is not very common in Palestine. Only about thirty specimens were found, mainly on the four species of *Rhinolophus* and on *Myotis myotis*. A single specimen was found on *Miniopterus schreibersi*. Speiser, Falcoz and Karaman record it from the same species of bats and also from *Rhinolophus mehelyi* and *Plecotus auritus*. Specimens from the following localities and hosts were examined: Austria (*Rhinolophus hipposideros*), Croatia (*Rhinolophus* sp.), Germany (*Plecotus auritus* and *Rhinolophus hipposideros*), Italy (*Miniopterus schreibersi* and *Rhinolophus ferrumequinum*), Morocco and Turkey (*Myotis daubentoni* and *Rhinolophus ferrumequinum*), Egypt (*Rhinolophus* sp.), Algeria (*R. ferrumequinum*).

*Nycteribia biarticulata* is distributed throughout Europe, including Britain and Spain, North Africa and extending eastwards into Western Asia.

7. *Nycteribia* (*Stylidia*) *biloba* n.sp.

Description. Length 3 mm. Colour reddish brown.

*Head* moderately compressed laterally, but rounded posteriorly, well sclerotized nearly up to the anterior margin, which bears 4 to 6 moderately long setae in the middle. A row of short hairs, which may be double, in the anterior part of the ventral margin. Posterior half with only one long and one short seta. 2 or 3 setae on the genae. Eyes absent. Labella of proboscis shorter than theca.

*Thorax* slightly wider than long. Length 1·15 mm. Width 1·3 mm. Median sternal suture well sclerotized, narrow, very slightly thickened but not flattened in the middle third. Oblique sutures joining median suture at an angle of about 75°. Ctenidium with 22 to 25 long, pointed teeth. Dorsal pattern in general similar to that of *N. biarticulata*. Notopleural sutures parallel, mesonotal space slightly less than one-third of width of thorax. Anterior spiracles elliptical, close to anterior end of sutures. Lateral plates of notopleural sutures short, wider than in *N. biarticulata*. Mesopleural sutures long, narrow and well sclerotized. A row of 7 to 10 notopleural hairs beginning further posteriorly than in *N. biarticulata*, slightly in front of the origin of the mesopleural sutures. Spaces between the anterior hairs much wider than between the posterior ones. Tibiae long and narrow, scalpel shaped, laterally compressed. Front tibiae nearly as long as femur, about 4·5 or

5 times as long as wide. Three transverse rows of moderately long, strong setae in the distal half of the tibiae. An area of small spines in the basal half of the anterior surface of the fore tibia, as in *N. biarticulata*, but the individual spines are larger.

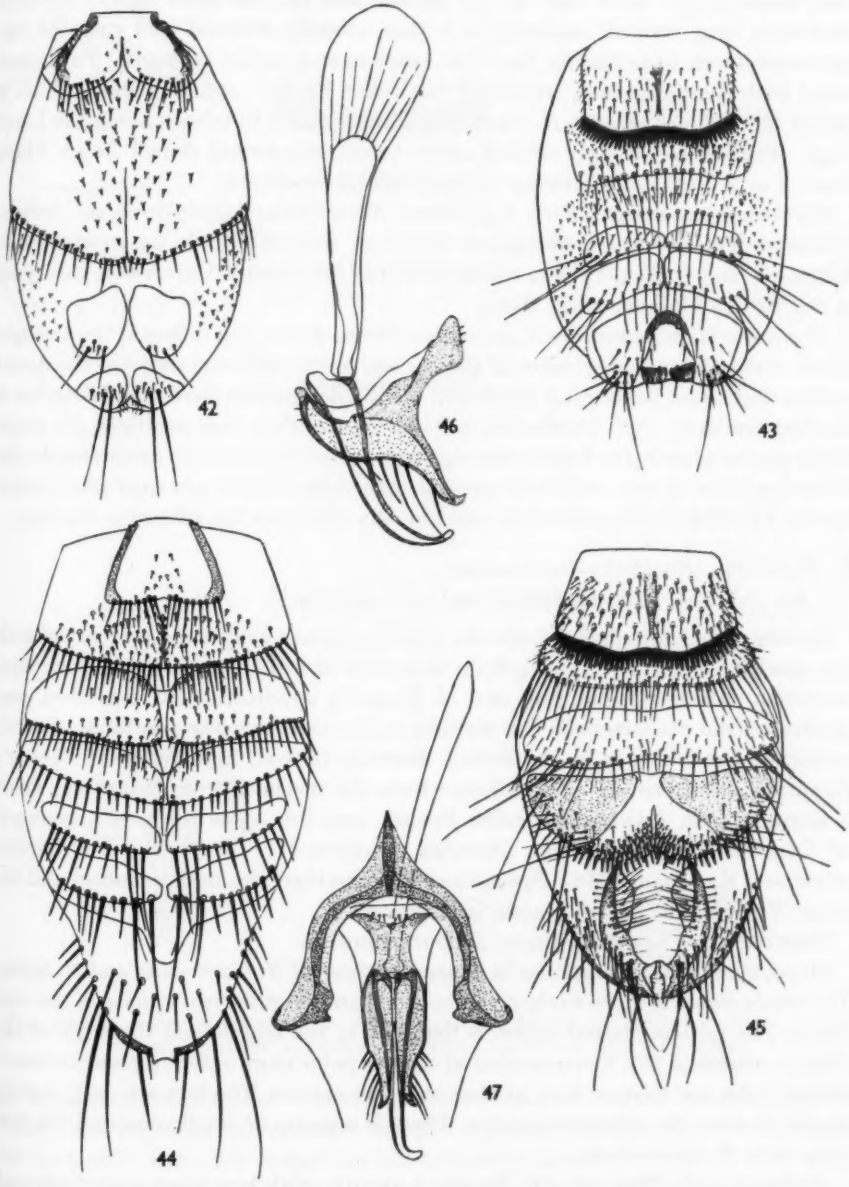
*Abdomen female* (Figs. 42, 43). Tergite 1 twice as wide as long, divided posteriorly into two lobes, each of which bears a row of moderately long setae at the posterior margin, leaving the indentation between the lobes bare. A few minute hairs in the middle of the tergite. Second tergal plate (probably consisting of the fused tergites 2 to 4) very large, as long as wide, slightly rounded posteriorly, and incompletely divided by a median line. At its posterior margin a row of moderately long setae laterally and some short spines in the middle. The surface of the tergal plate is covered with short spines in the middle and bare at the sides. The area of tergites 5 and 6 is membranous. In its middle two sclerites, rounded posteriorly and bearing one or two longer and a few shorter setae. The sides of the abdomen are covered with small spines. Anal tergite short, divided into two halves, each bearing two very long setae at the tip and some short setae in front of them.

Sternite 1 + 2 rectangular, narrower than the abdomen, with a ctenidium of 50 to 55 teeth and 4 to 5 rows of short hairs in the posterior half. Postspiracular sclerite as in *N. biarticulata*. Sternites 3 and 4 membranous, with a row of longer setae posteriorly and 2 to 3 rows of small setae anteriorly. Sternites 5 and 6 divided into lateral plates which stand close together and each of which bears a row of long setae posteriorly, the lateral setae being the longest. A row of short setae in the middle of the plates. Sternite 7 divided into two triangular plates which protrude posteriorly. They bear 2 transverse rows of short setae in the middle and a double row of 6 to 7 long setae at the tip on each side. The rows of setae continue into the space between the plates. Dorsal genital plate shield-shaped, similar to that of *N. biarticulata*, but larger, with rounded anterior margin and a row of 8 to 10 small hairs at the posterior margin. Anterior margin and two rectangular areas at the posterior margin pigmented. Size: 0·35 × 0·27 mm. (Fig. 39*b*). Ventral genital plate absent.

*Abdomen male* (Figs. 44, 45). Tergite 1 triangular with a shallow indentation at the posterior margin, and hairs as in female. Tergites 2 and 3 with straight hind margin which bears a uniform row of moderately long hairs. Both tergites are covered with small hairs except at the sides. Tergite 4 narrower, with a similar row of setae posteriorly and a group of small hairs in the middle. Tergites 2 to 4 are incompletely divided in the middle. Tergites 5 and 6 are divided into narrow lateral sclerites, each of which has a row of long and short setae posteriorly, the 2 to 4 median setae being the longest. Surface bare. Anal segment conical, not very wide, with several transverse rows of short hairs and some long setae posteriorly.

Sternite 1 + 2 similar to that of the female. Sternite 3 narrow, with a row of long and short setae posteriorly and 2 to 3 rows of short hairs on the surface. Sternite 4 similar but wider, with 2 to 3 rows of short hairs on the surface in the posterior half and the lateral setae of the posterior row very long. Sternite 5 still wider, with two broad lobes posteriorly, each of which is covered with 4 to 5 rows of short spines which becomes progressively longer posteriorly. Some long setae

and some short hairs at the sides of the segment. Anterior part of the surface bare. Two rounded bulges covered with hairs in the anterior part of the sides of the ventral surface of the anal segment.




Figs. 42–47. *Nycteribia biloba*. 42, female, abdomen, dorsal; 43, female, abdomen, ventral; 44, male, abdomen, dorsal; 45, male, abdomen, ventral; 46, male genitalia, profile; 47, male genitalia, ventral.

*Genitalia* (Figs. 46, 47). Similar in general to those of *N. biarticulata*. Basal arc broad, pigmented, with moderately long anterior process and lateral flaps posteriorly. Claspers thick, curved, tapering to a darkly pigmented point, with long setae in the basal half on the outside and shorter hairs further distally. Aedeagus long, curved, tapering to a thin, slightly widened and rounded tip. Apodeme short, wide basally, less than twice as long as the aedeagus. Parameres fused with the phallobase, with long, beak-like, hooked, apical process, which is longer and thinner than in *N. biarticulata*, and bears 5 to 6 hairs along the lower edge. Phallobase strongly arched, with broad, pigmented dorsal hinge which bears 6 to 8 small hairs between its base and the basal arc.

MATERIAL EXAMINED. 5 ♀♀, 4 ♂♂ from *Rhinolophus hipposideros*, *R. ferrum-equinum*, *Miniopterus schreibersi* and *Myotis* sp. from Herzlia in the coastal plain, Jerusalem and Galilee. Type in the collection of the Hebrew University, paratypes in the British Museum (Nat. Hist.).

*Nycteribia biloba* resembles *N. scissa* from South Africa, but differs in the arrangement of the abdominal sclerites of the female, in the different shape of the dorsal genital plate, and in the male genitalia. As the description shows, *N. biloba* has a marked similarity to *N. biarticulata* in the structure of the male genitalia, the shape of the genital plate in the female, the segmentation of the abdomen in the female, the structure of the thorax, and in the presence of a characteristic group of small spines on the fore tibia, and is therefore provisionally placed in the subgenus *Stylidia*.

8. *Nycteribia* (*Stylidia*) *integra* n.nom.

For *Nycteribia blainvillii* Speiser, 1901 (nec Leach 1817).

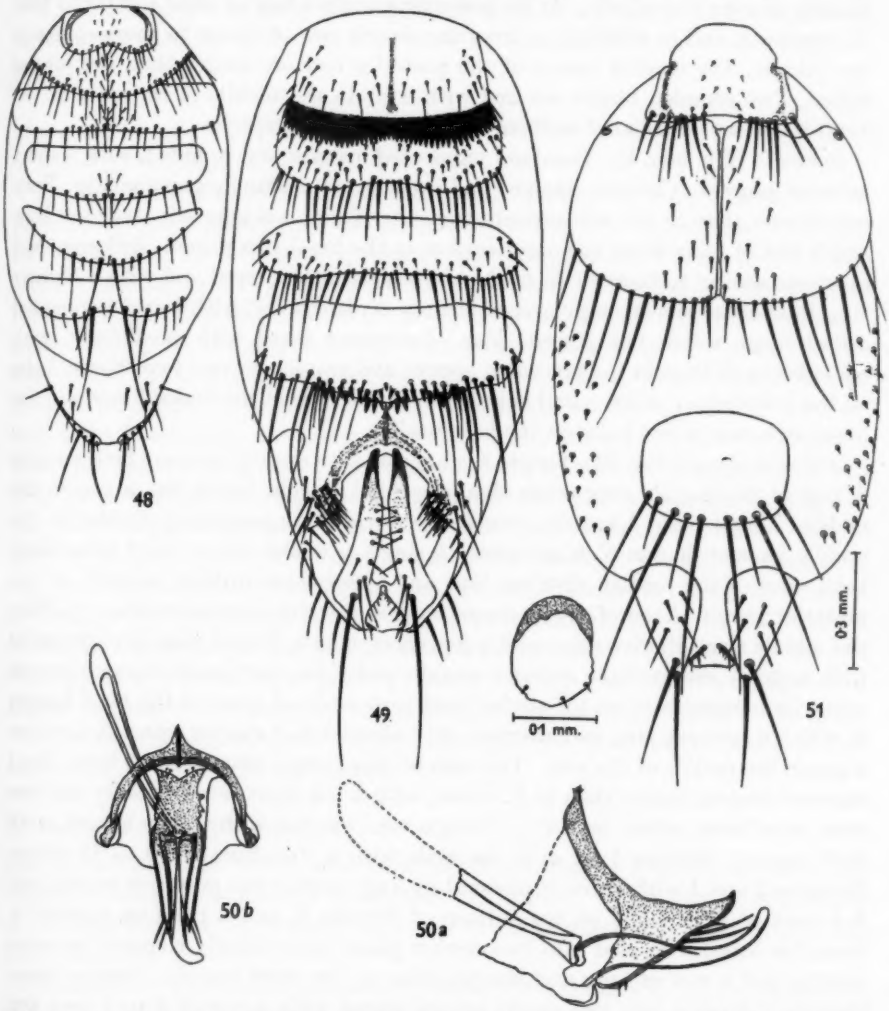
Speiser (1901) described this species from Egypt and considered it identical with the species described by Leach from Mauritius in 1817 because of its long legs. Karaman (1939) re-examined one of Speiser's specimens and described and illustrated the characteristic 5th sternite of the male and the genitalia. Examination of Leach's type in the British Museum proved, however, that Speiser's Egyptian specimens are quite different from the specimen from Mauritius, which is identical with *Tripselia amiculata* Speiser, and this name becomes a synonym of *Tripselia blainvillii* Leach. (Another synonym of *T. blainvillii* is *Nycteribia reichenowi* Karaman, 1948.) Speiser's species has therefore to be renamed and the name *N.* (*Stylidia*) *integra* n.nom. is proposed.

DESCRIPTION. Length 2·5 mm. Colour yellowish.

Head, thorax and legs similar in general to those of *N. biarticulata* and *N. biloba*. The whole insect is more weakly sclerotized than the other two species of the subgenus. The median sternal suture is flattened in the middle and the angle of the oblique sutures is 75°. The mesopleural sutures point more anteriorly and the notopleural hairs are thinner than in the other two species. The legs are only slightly longer than in the other two species. There is a group of small spines on the fore tibia as in *N. biarticulata*.

*Abdomen male* (Figs. 48, 49). Tergite 1 narrow, with two lobes posteriorly and a row of about 15 moderately long hairs at the posterior margin. Tergite 2 rounded posteriorly, with a row of uniformly long setae at the posterior margin and a small

group of short hairs in the middle. Tergites 2 to 4 with traces of a median dividing line. Tergites 3 to 6 narrow, slightly convex posteriorly, with similar rows of setae, except for a pair of longer setae in the middle of tergites 5 and 6. A group of about




Figs. 48–51. *Nycteribia integra*. 48, male, abdomen, dorsal; 49, male, abdomen, ventral; 50, male genitalia: *a*, profile; *b*, ventral; 51, female, abdomen, dorsal.

10 small hairs in middle of tergite 3 and a few hairs on tergites 4 and 5. Tergite 6 bare. Anal segment conical, bare, except for a transverse group of about 6 hairs in the middle and a few longer and short hairs posteriorly.

Sternite 1 + 2 rectangular with a ctenidium of 45 long teeth, and 2 to 3 rows of short hairs in the posterior half. Postspiracular plate similar to that of *N. biarticulata*. Sternites 3 and 4 narrow with a row of moderately long hairs at the posterior

margin. Some of the lateral setae longer than the others. 2 or 3 rows of short hairs on the surface of sternite 3. Sternite 4 bare except for 1 or 2 rows of short hairs before the posterior row. Sternite 5 longer than the preceding sternites and slightly convex posteriorly. At its posterior margin a row of setae similar to that of sternite 4, and in addition an irregular double row of about 25 short spines in the middle. The median spines of the posterior row are longer than the lateral spines. Two rounded bulges covered with hairs in the middle of the sides of the ventral surface of the anal segment as in the preceding species.

*Genitalia* (Fig. 50*a*, *b*). Basal arc pigmented, narrow and rounded, with a short anterior process. Claspers straight and tapering to a blunt pigmented tip. They are thinner than in the two preceding species. There is a long seta near the base and a row of hairs along the outer surface in the basal two thirds. Aedeagus and apodeme similar to those of *N. biloba* but less strongly curved and with a blunter tip. Phallobase less strongly arched than in *N. biloba* and with broad pigmented dorsal hinge which has jagged sides. Parameres fused with phallobase, with a shorter beak than in the preceding species and two longer and two shorter hairs on the lower edge. A few small hairs between the base of the dorsal hinge and the basal arch, and a few hairs on the base itself.

*Abdomen female* (Fig. 51). Tergite 1 triangular with slightly concave hind margin. A row of moderately long setae along the hind margin which has a gap in the middle. Tergal plate 2 broadly rounded posteriorly, incompletely divided in the middle, shorter than in *N. biarticulata*. A single or double row of short setae along both sides of the median dividing line, and a few setae further laterally at the posterior margin. A row of widely spaced long setae which are shorter laterally. Near the middle some shorter setae and a few short spines. Tergal plate 3 elliptical or with more or less straight anterior margin and sides, and more strongly curved posterior margin, bare on the surface and with a row of setae at the hind margin of which 4 are very long and alternate with about 4 to 6 shorter setae. Sometimes a gap in the middle of the row. The area between tergal plate 2 and 3 bare. Anal segment conical, longer than in *N. biloba*, with a few short setae dorsally and two short anal lobes, which bear 2 to 3 long setae. Pleurae with widely spaced, very short spines. Sternite 1+2 as in the male with a ctenidium of 50 to 55 spines. Sternites 3 and 4 with a row of moderately long setae at the posterior margin and 3–4 rows of short setae on the surface of sternite 3, and 2 rows on sternite 4. Sternites 5 and 6 divided into two narrow plates with slightly concave posterior margin and a row of long and shorter setae at the hind margin. Surface bare. Sternite 7 divided into two nearly square plates, with a row of 5 to 6 long and shorter setae posteriorly and a transverse row of 3 to 4 short setae in the middle of the surface. Anal segment bare ventrally, except for two setae near the anus. There is a small triangular anal sclerite, which bears two short setae. This consists sometimes of a single seta. Genital plate similar to that of *N. biarticulata* (0·14 × 0·1 mm.) with irregularly rounded posterior margin and 3–4 very short hairs near it.

MATERIAL EXAMINED. 1 ♂ from *Rhinolophus blasii*, Southern Judaea and 1 ♂ from *Rhinolophus* sp., Jerusalem. Eighty-five specimens, Yemen, Arabia from

*Rhinolophus acrotis acrotis, R. blasii, Hipposideros caffer* (Coll. H. Hoogstraal). Eighty-nine specimens, Egypt, from *Rhinolophus acrotis acrotis, R. acrotis brachygnathus, Rhinopoma hardwickei cystops* (Coll. H. Hoogstraal).

*Nycteribia integra* shows a marked resemblance to the two preceding species in all essential details and is therefore also provisionally placed in the subgenus *Stylidia*.

## Genus **Basilia** Mir. Ribeiro, 1903

Type species: *Basilia ferruginea* Mir. Rib. 1903.

The genus was created for a South American species. Its main character is the presence of eyes consisting of two incompletely divided ocelli with a pigmented basis (Fig. 52). All American species of Nycteribiidae have subsequently been found to belong to this genus. Only a few species of the genus are known from the Old World. Except for the structure of the eyes, there are nearly no characters which distinguish the genus clearly from either *Penicillidia* or *Nycteribia*. The structure of the eye is sometimes insufficient for diagnosis, as in some specimens the eyes are single, or single on one side and double on the other. Furthermore, eyes of the *Basilia* type are also present in the genus *Paracyclopodia*. The genitalia have been studied only in the three species dealt with in the present paper, and in general resemble those of the genus *Nycteribia*. The dorsal genital plate of the female is much reduced in the Old World species examined, and the ventral plate is also very small or absent. On the other hand, the figure of the genital region of a female of an American species given by Guimarães (1946), and that of an unidentified species examined, show that the arrangement of the sclerites ventral to the anus and the genital plate differs markedly from that in the Old World species. Species of the genus *Tripselia* resemble some Old World species of *Basilia* in many respects except for the absence of eyes. A definition of the genus will have to be postponed until the genitalia of the American species and of further Old World species have been studied.

### 9. *Basilia nattereri* Kolenati, 1857

This species has been clearly described and illustrated by Kolenati in 1863, and a specimen from the collection of the Museum in Paris, which may be considered as the type collected by Kolenati and kept in his characteristic small bottle, has been examined.

Examination of material of *B. nattereri* from Palestine and Europe showed that there are two species, both of which have been considered as *B. nattereri* by previous authors. There is a larger species which apparently is the true *B. nattereri* as Kolenati's specimen in Paris belongs to this species. His figure shows the chaetotaxy of this species and he gives its size as 3 mm., while the second species which will be described below as *Basilia nana* n.sp., rarely measures more than 2·2 mm. The specimen described by Karaman as *B. nattereri* belongs to this latter species, while Speiser apparently had both species before him. Both are closely related, but apart from size, differ also in several characters chaetotaxy and in the structure of the male genitalia.

DESCRIPTION. Length 3 mm. Colour light brown.

*Head.* Laterally compressed. Double eyes with rather long cylindrical pigmented base near the anterior dorsal margin of the head. The posterior opening of the ocular sclerite is as wide as the whole sclerite (Fig. 52*c*, *g*). Between the eyes

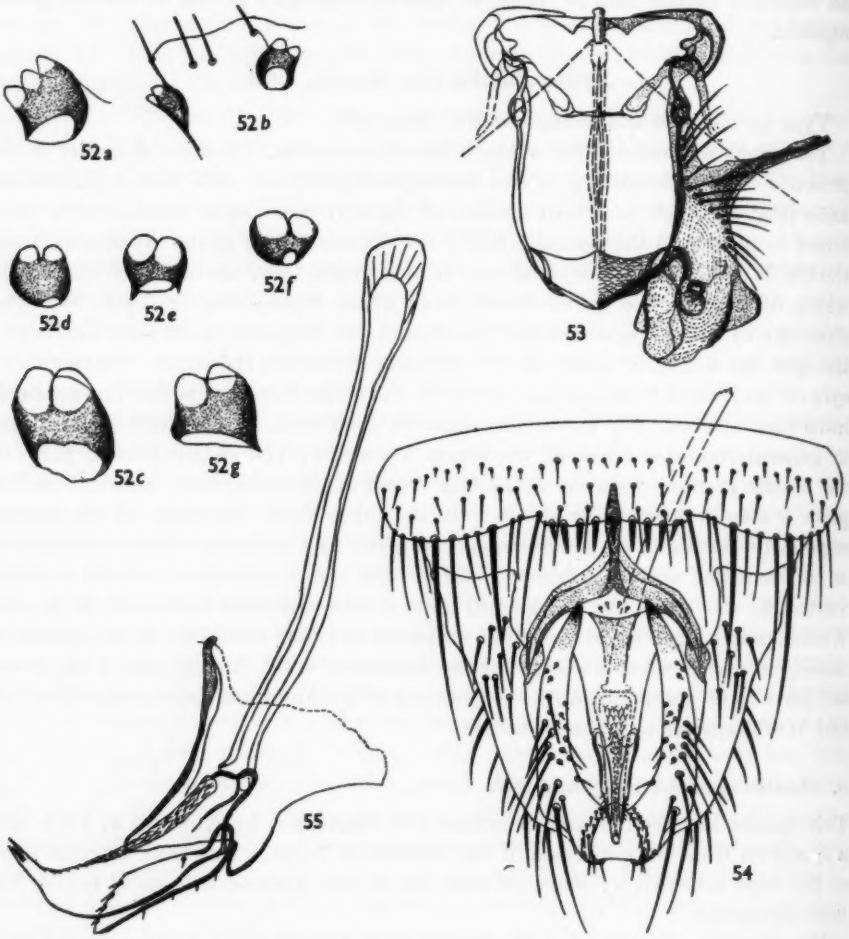



Fig. 52. Eyes of species of *Basilia*. *a*, *b*, *B. bathybothyra daganiae*; *c* and *g*, *B. nattereri*; *d–f*, *B. nana*. Figs. 53–55. *B. nattereri*. 53, dorsal pattern of thorax; 54, male, sternite 5 and genital area; 55, male, genitalia, profile.

a row of 6 setae of moderate length. This row continues with shorter setae along the anterior ventral margin of the head. Some 6 to 8 short setae on the genae. Labella of proboscis shorter than the theca.

*Thorax* wider than long. Length 1 mm. Width 1·3 mm. Dorsal pattern (Fig. 53): Mesonotal space wide, rounded posteriorly. Anterior spiracles round, at level of posterior edge of insertion of head. Lateral plates of notopleural sutures broad,

mesopleural sutures long and narrow. About 15 notopleural hairs in S-shaped row beginning a short distance behind the spiracle. Median sternal suture narrow, slightly widened in middle. Sutures between meso- and metasterna joining median suture at right angles. Tibiae flattened, rather slender, scalpel-shaped, 3·5 times as long as broad. 3 transverse rows of strong setae distally. Thoracic ctenidium with about 25 long pointed teeth.

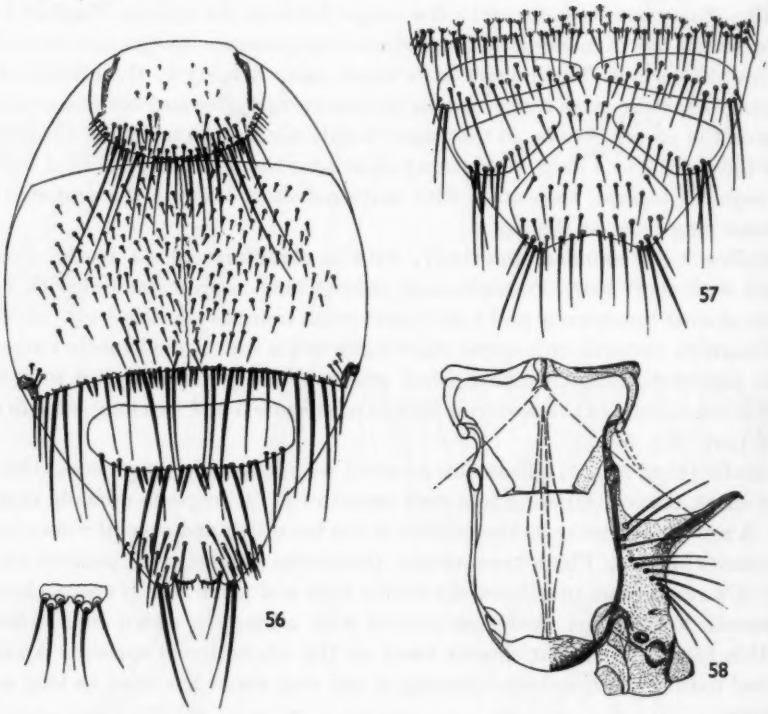
*Abdomen male.* Tergite 1 twice as wide as long, shallowly rounded posteriorly with a small indentation in the middle of the posterior margin. On the posterior margin a double row of moderately long setae which become gradually shorter laterally. Some minute hairs and a few longer hairs on the surface. Tergites 2 to 6 narrow with a row of moderately long setae at the posterior margin and some short hairs on the surface. These short hairs stand more densely in the middle of the segments. They are more numerous on the anterior tergites and become gradually less so on the posterior ones, so that there is only one row on tergite 6. On tergites 4 to 6 there are 2 to 4 long, very strong setae near the middle of the hind margin. Anal segment conical, truncated, with scattered short setae in the posterior half and some long setae at the tip.

Sternites 1+2 rounded anteriorly, with a ctenidium of 55 teeth. Surface covered with short hair. Postspiracular sclerite long, narrow, curved with 1 to 2 long setae near the spiracle and 4 to 6 short setae towards sternite 1+2. Sternites 3 to 5 narrow, covered with sparse short hairs and a row of moderately long setae at the posterior margin which become gradually longer laterally. 6 to 8 short spines in the middle of the posterior margin of sternite 5 and two long setae in each lateral part (Fig. 54).

*Genitalia* (Figs. 54, 55). Basal arc rounded with long anterior process. Claspers rather short, curved, tapering to a dark smooth tip. A long seta dorsally near the base. A row of long setae on the outside in the basal half and several rows of small hairs nearer the tip. Phallobase conical, parameres slender, with pointed tip and a row of 4 small hairs near it on the dorsal edge and some widely spaced hairs at the smooth lower edge. Aedeagus curved with a long tip which ends in several long thin teeth and similar shorter teeth on the whole dorsal surface. Apodeme long and narrow, with a short widening at the end, about 2·5 times as long as the aedeagus.

*Abdomen female* (Figs. 56, 57). Tergite 1 similar to that of male, but without an indentation in the posterior margin and with the setae much longer and the hairs of the posterior part of the surface longer and more numerous. Tergites 2 to 5 fused into a broad rectangular plate, nearly twice as wide as long. Its surface is covered with short hairs which become gradually longer posteriorly, except for narrow, bare stripes at the sides. Posterior margin slightly concave with a row of long setae and some short spines between them in the middle. At the posterior corners a small angular plate in which the lateral seta is inserted. In front of this plate 3 short spines. Tergite 6 bare, with a row of 8 to 10 long setae and short spines among the long setae in the middle of the posterior margin. Anal segment short, covered with moderately long setae in the posterior part and with some long setae at the tip.

Sternite 1 + 2 as in the male, with a ctenidium of 58 to 62 long teeth. Sternites 3 and 4 indistinct, densely covered with hairs and with rows of longer hairs indicating their posterior margin. Sternite 5 divided into narrow elliptical halves, covered with short setae and some longer setae at the sides. Sternite 6 divided into triangular halves with one row of setae which are longest at the sides and with some hairs between the plates. Sternite 7 triangular, covered with hairs of medium length and with a row of long setae at the posterior margin. Dorsal genital plate small, with two processes which each bear 2 to 3 moderately long hairs. Ventral plate not discernible.




Figs. 56–57. *Basilia nattereri*. 56, female, abdomen, dorsal and genital plate; 57, female, posterior part of abdomen, ventral. Fig. 58. *B. nana*. Dorsal pattern of thorax.

MATERIAL EXAMINED. 1 ♀ from Kolenati's collection in the Museum in Paris, which is considered as the type. 1 ♂, 1 ♀ from Anger, France, from *Eptesicus serotinus*, 27 February 1883. 1 ♂, Crimea, no further data.

10. *Basilia nana* n.sp.

This species has not been previously separated from *B. nattereri*, from which it differs in size, chaetotaxy and the structure of the male genitalia.

DESCRIPTION. Length 1·8–2·2 mm. Colour yellowish.

*Head* laterally compressed, frons forming a sharp angle between the eyes. 2 setae between the eyes on the anterior margin of the head. A row of widely

spaced short hairs along the anterior ventral margin of the head. A group of 4 to 6 hairs on the genae. Eyes double, on rounded, pigmented base with small posterior opening (Fig. 52*d–f*). Labella of proboscis shorter than the theca.

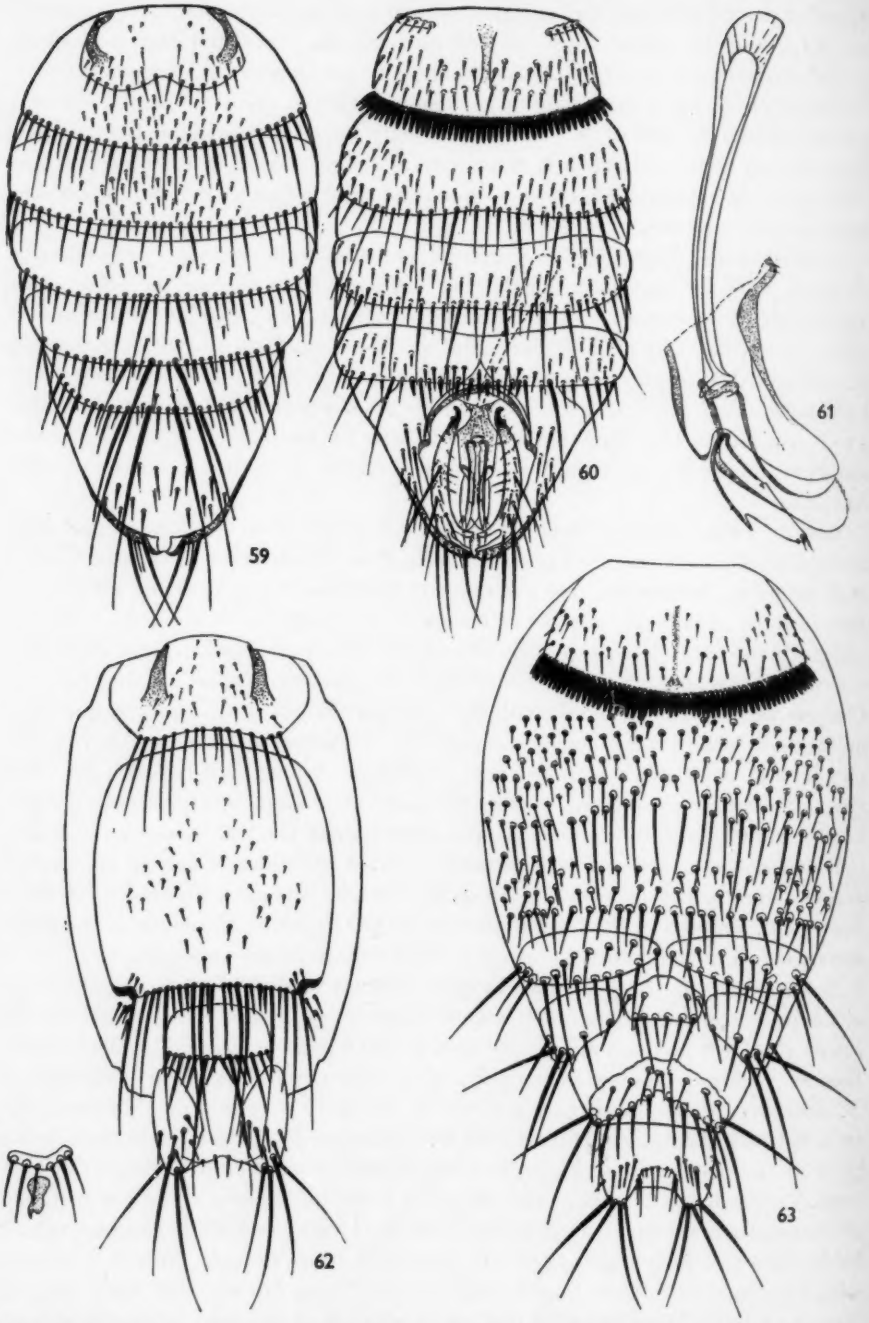
*Thorax* (Fig. 58) wider than long. Length 0·7 mm. Width 0·9 mm. Dorsal pattern similar to that of *B. nattereri*, but only 9 to 12 notopleural hairs, which begin further posteriorly than in *B. nattereri*. Median sternal suture flattened and widened in the middle half. Thoracic ctenidium with about 20 pointed long teeth. Legs as in *B. nattereri*.

*Abdomen male* (Figs. 59, 60). Tergite 1 twice as wide as long with shallowly rounded posterior margin which has an indentation in the middle. A row of widely spaced short hairs at the posterior margin and an irregular double row of similar hairs in front of it. These hairs are less numerous and shorter than in *B. nattereri*. Tergites 2 to 6 narrow, with moderately long and short setae at the hind margin and 2 to 4 very long setae in the middle of the hind margin of tergites 4 to 6. Surface of tergites 2 to 4 with short hairs in the middle, which become less numerous on the posterior tergites. Only 4 to 6 hairs on tergite 5 and two or none on tergite 6.

Sternite 1 + 2 rounded anteriorly with a ctenidium of 50 to 55 teeth and with 3 to 4 rows of short hairs in its posterior half. Postspiracular sclerite similar to that of *B. nattereri*, but shorter and less curved. Sternites 3 and 5 narrow with 2 to 3 rows of short hairs on the surface and a row of long and short hairs at the posterior margin. 6 to 8 short, strong spines in the middle of the posterior margin of sternite 5.

*Genitalia* (Fig. 61). Basal arc rounded or triangular with short anterior process. Claspers rather short, curved, with dark tip turned outwards and a strong subapical tooth, with a row of long setae and some shorter setae in the basal half on the outside and with short hairs further anteriorly. A long seta dorsally near the base. Phallobase cylindrical, parameres narrowly triangular with a strong tooth on the lower edge and two longer and a short hair at the tip. Apodeme long and slender. Aedeagus curved with broadly rounded tip which is curved backwards and has some serrations near the tip on the outside. The genitalia thus differ from those of *B. nattereri* in the form of the tip of the clasper, in the shape of the parameres, which have a ventral tooth, and in the shape of the aedeagus.

*Abdomen female* (Figs. 62, 63). Tergite 1 similar to that of male, but the setae at the posterior margin are much longer. Tergites 2 to 5 fused into a broad, nearly square plate with a diamond-shaped field of small hairs in the middle. These hairs become gradually longer posteriorly. They are much less numerous than in *B. nattereri*. At the hind margin a row of about 10 long setae with some short setae between them, mainly in the middle. The lateral seta is inserted in the angular plate at the posterior lateral angle of the tergite. Just in front of it 2 to 3 short hairs. Tergite 6 half as wide as the preceding plate, quite bare, with a row of 4 long and several short setae at the posterior margin. Anal lobes with 2 to 3 rows of short hairs and 4 to 5 long setae at the tip. Sternite 1 + 2 as in male. Sternites 3 and 4 with several rows of small hairs and a row of long hairs at the hind margin. Sternite 5 divided into two elliptical halves which bear two rows of setae which are longest in the posterior row at the sides. Sternite 6 narrow, arc-shaped with two




Figs. 59–61. *Basilia nana*. 59, male, abdomen, dorsal; 60, male, abdomen, ventral; 61, male, genitalia, profile. Figs. 61, 62. *B. nana*. 61, female, abdomen, dorsal and genital plates; 62, female, abdomen, ventral.

triangular sclerites at the sides which bear one row of setae posteriorly and a median triangular sclerite which bears two rows of setae. Sternite 7 narrow with rounded anterior and concave posterior margin bearing a row of long setae posteriorly and a row of 6 to 8 hairs on the surface. Dorsal genital plate small, of irregular triangular shape with 4 to 6 long hairs at the posterior margin. Ventral plate with convex posterior surface, narrow, with rounded anterior margin.

*B. nana* is closely related to *B. nattereri* from which it differs in its much smaller size, in the structure of the eyes, the male genitalia, the segmentation of the abdomen in the female and in some details of chaetotaxy. European material differs from Palestinian material in its slightly larger size and in the shape of the ocular sclerite which has a wider posterior opening.

Hosts. *B. nana* was found commonly on *Myotis nattereri* (120 specimens) and only a few on *M. myotis*. Karaman mentions a specimen of *Basilia nattereri* from *Plecotus auritus* in Germany. This is definitely *Basilia nana* according to the structure of the male genitalia. Speiser gives *Myotis nattereri*, *M. daubentoni*, *M. myotis* (?) and *M. bechsteini* as hosts, and states that the specimens from *M. myotis* and *M. bechsteini* were much smaller. They were thus probably *Basilia nana*.

A specimen was examined from *Plecotus auritus* or *Rhinolophus hipposideros* from Germany (Coll. Vienna Museum) and four specimens from Stuttgart, Germany (Coll. Zoological Museum, Stuttgart), a specimen from *Myotis mystacinus* from Rouen, and a specimen from Kolenati's collection in the Paris Museum. Several specimens from Holland from *M. dasycneme*, *M. emarginatus* and *M. bechsteini* (Museum Amsterdam) were examined. Some specimens from Ludlow, Shropshire, Great Britain, found on *M. bechsteini* (3 September 1953) were examined (Coll. Blackmore). This is the first record of *Basilia nana* from Great Britain. Type and paratypes in the collection of the Hebrew University, paratypes in the British Museum (Nat. Hist.).

Distribution. The species thus seems to occur rarely in Britain, France, Germany and Holland, but is rather common in Palestine. It was found most frequently on *Myotis nattereri* in July and August (5 to 10 per bat) and in small numbers throughout the year. On *M. myotis* it was found in small numbers in July and August only.

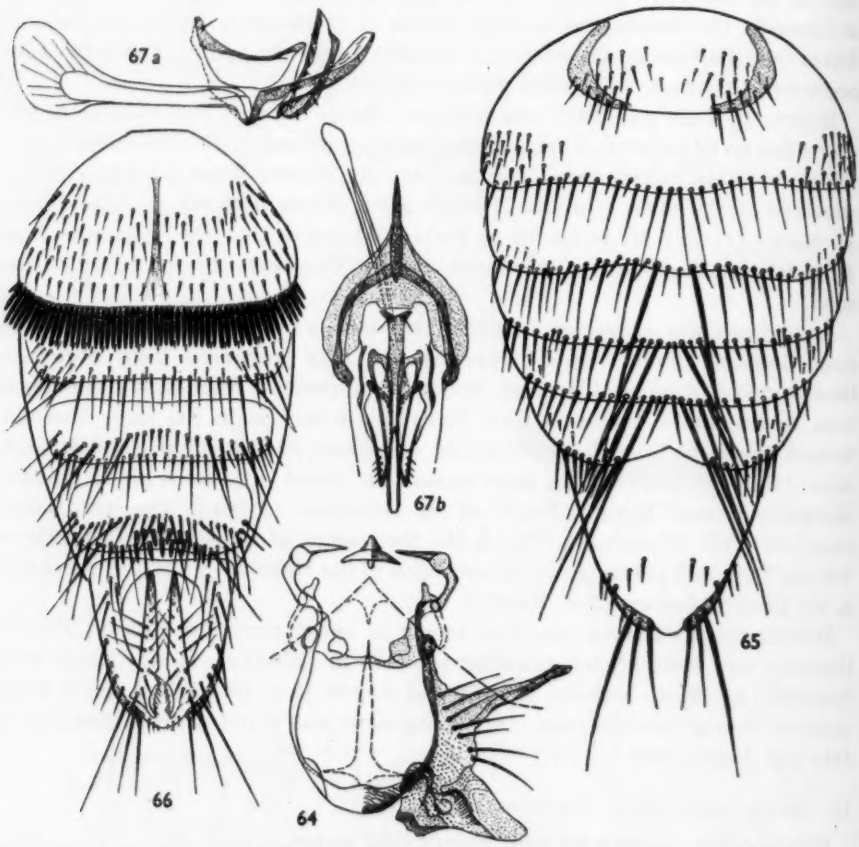
11. *Basilia bathybothyra daganiae* n.subsp.

Description. Length 2·5 mm. Colour light brown.

*Head* moderately compressed laterally. Eyes with double, sometimes triple ocelli and broad pigmented base. Posterior opening of the ocular sclerite wide. The eyes are situated close to the anterior margin of the head and protrude from the surface (Fig. 52*a*, *b*). 4 setae between the eyes and a row of short setae along the anterior ventral margin of the head. A long seta at the basal end of the row and a few short hairs on the genae. Labella of proboscis about a third of the length of the theca.

*Thorax* (Fig. 64) wider than long. Length 0·85 mm. Width 1 mm. Dorsal pattern: mesonotal space widening posteriorly. Anterior spiracle round, situated

at posterior limit of insertion of head. Lateral plate of notopleural sutures broad. The row of 7 to 9 notopleural hairs begins some distance behind the spiracles. Median sternal suture narrow and well-marked posteriorly, but indistinct anteriorly. Oblique sutures clearly marked, joining median suture at an angle of about 95°. Thoracic ctenidium with about 20 long pointed teeth. Tibiae long and slender, 4·5 times as long as wide, with 3 rows of relatively short setae in the distal third quarter.




Figs. 64–67. *Basilia bathybothyra daganiae*. 64, dorsal pattern of thorax; 65, male, abdomen, dorsal; 66, male, abdomen, ventral; 67, male genitalia: *a*, profile; *b*, ventral.

*Abdomen male* (Figs. 65, 66). Tergite 1 small, rounded, pigmented at the sides, with a few short hairs at the sides of the posterior margin and a few shorter hairs on the surface in the lateral posterior area. Tergite 2 large, slightly convex posteriorly. Tergites 3 to 6 narrow. All tergites bare except for groups of short hairs at the sides of tergite 2 and a few very small hairs at the sides of tergites 3 to 6. All tergites with rows of moderately long and short setae at the posterior margin and 2 to 4 very long setae in the middle of the posterior margin of tergites 3 to 6.

A wide concave gap without hairs in the middle of the hind margin of tergite 6. Anal tergite conical, bare dorsally, except for a transverse row of 5 to 6 hairs and a few hairs at the sides. Several long setae at the tip.

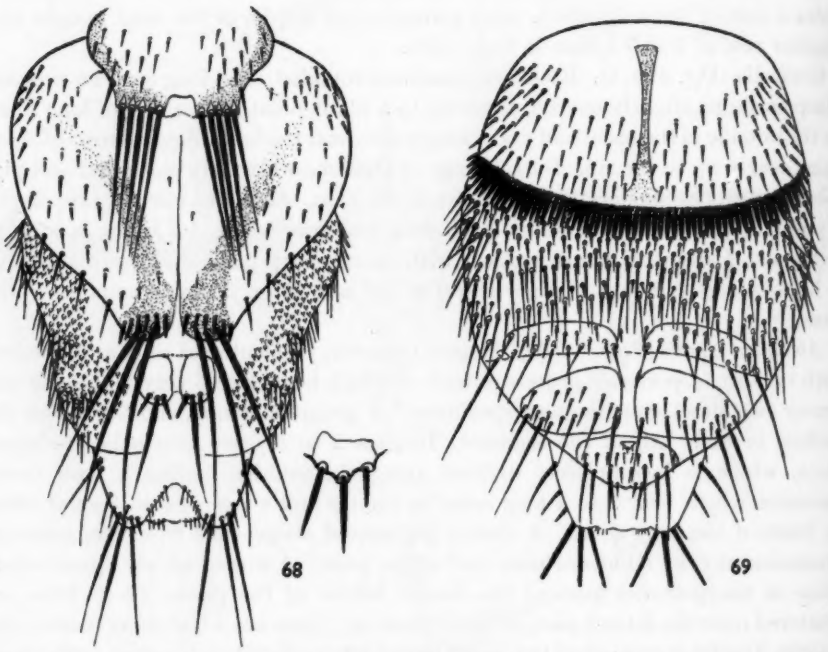
Sternite 1+2 rounded anteriorly with a ctenidium of about 60 very long teeth. Surface covered with small hairs except in anterior middle part. Postspiracular sclerite wide and short, triangular, with 2 very long setae near the spiracle and 3 to 4 short setae towards sternite 1+2 (Fig. 5*d*). Sternites 3 to 5 narrow, with a row of moderately long and shorter setae at the hind margin, 2 to 3 rows of short hairs on the surface of tergite 3 and one row on tergites 4 and 5. 1 or 2 long setae at the sides of the segments. In addition to the rows of seta, sternite 5 bears a row of 10 to 12 short, thick spines in the middle of the hind margin and another row of 6 to 7 spines in front of it.

*Genitalia* (Fig. 67*a*, *b*). Basal arc broad and rounded, with long anterior process. Claspers short, slightly curved, tapering to a blunt point with a row of long setae on the outside in the basal half and a longer seta near the base. Several rows of short hairs nearly up to the tip. Dorsal hinge of phallobase strongly chitinized and pigmented with two moderately long hairs at the base. Aedeagus curved, tapering to a point. Apodeme rather short, with wide posterior plate, 1·5 times as long as aedeagus. Parameres parallel sided, with short, sharp point and a row of about 5 hairs along the dorsal edge near the tip and 2 or 3 small hairs near the base.

*Abdomen female* (Figs. 68, 69). Tergite 1 narrow, pigmented at sides and rounded with two broad posterior processes, each of which bears 3 to 5 very long setae and one or two short setae in some specimens. A group of strong, short setae on the surface in each half of the segment. Tergites 2 to 5 fused into a heart-shaped plate, which is incompletely divided along the middle, ending in two broad processes which bear 2 to 4 long setae at the tip and a row of 5 to 8 short setae in front of the long setae. A darkly pigmented stripe runs from the posterior processes into the middle of each half of the plate. A row of widely spaced short setae at the posterior part of the lateral border of the plate. Short hairs are scattered over the lateral part of the surface and there are a few short hairs in the middle. Tergite 6 consists of two small irregularly rounded plates, each with a long and 2 or 3 short setae posteriorly. The sides of the abdomen are covered with small spines on raised tubercles. Anal segment bare, with a few setae at the sides and 1 or 2 longer and a few shorter setae at the tip.

Sternite 1+2 similar to that of male with a ctenidium of 65 to 70 teeth which are longer than in other species. Sternite 3 with a uniform row of hairs of medium length posteriorly and covered with short hairs which are longer at the sides. Sternite 4 narrow with a similar row of hairs at the hind margin, a double row of short hairs at the sides, but only one row in the middle. Sternites 5 and 6 divided into elliptical halves, each with 2 or 3 rows of short hairs and some longer setae at the sides of the hind margin. Sternite 7 rounded posteriorly with scattered short hairs and some longer setae at the hind margin. Dorsal genital plate small, irregularly triangular with 2 or 3 short hairs. The anal sclerite, which is present in other subspecies, is either absent or indicated by a single seta.

HOSTS. This species was found in small numbers on *Pipistrellus kuhli* in Dagania near the southern end of the Lake of Galilee. Only ten specimens were found on over 300 bats (Coll. Palmoni). This was the only species of nycteribiid found on this bat, which roosts under roofs and not in caves. Other specimens from Cyprus, also from *P. kuhli*, were examined. Type in the collection of the Hebrew University. One paratype in the British Museum (Nat. Hist.). Some specimens of *Basilia bathybothyra daganiae* from Egypt (Dakhlia Distr. on Pipistrellus Kuhli, 26.2.47), from the collection of the Chicago Natural History Museum, were also examined.




Figs. 68, 69. *Basilia bathybothyra daganiae*. 68, female, abdomen, dorsal and dorsal genital plate; 69, female, abdomen, ventral.

*B. bathybothyra* was originally described by Speiser from Calcutta (1907). Scott (1914) described *Penicillidia fletcheri* var. *pumila* from Ceylon, which later proved identical with *Basilia bathybothyra*. Later, the species was recorded from Africa. Examination of material from India (Bombay), Ceylon, Java and Africa showed that the various local forms differ in a number of details of chaetotaxy, in the form of the genital plate of the female and the anal sclerite. The male genitalia of the Mediterranean form resemble those of the Ceylon form more closely than those of the Java form. No males of the African form were available for study. The various forms are best regarded as subspecies for the time being. They will be dealt with in a paper on African Nycteribiidae which is in preparation.

## Genus **Penicillidia** Kolenati, 1863

Type species: *Penicillidia dufourii* Westwood, 1835.

This genus has been redefined by Speiser, who considered the presence of single eyes as the main character which distinguishes it from *Nycteribia*. The other genera possessing single eyes are sufficiently determined by other characters. In addition to the three European species, several tropical species were examined and, using some of the above-mentioned additional characters, the genus may be defined provisionally as follows:

Medium-sized or large insects. Head rounded posteriorly, not compressed laterally, with single, unpigmented eyes. Palps thick, with several dense rows of long and short setae (Fig. 2*b*). Thorax much broader than long. Mesonotum wide, narrowing posteriorly. Anterior spiracles slit-like and situated near anterior end of suture. Mesopleural sutures long and narrow. Oblique sutures indistinct. Notopleural setae either numerous or few or absent. Haltere groove with a protective cover. Thoracic ctenidium with narrow pointed teeth. Legs long, with long, scalpel-shaped tibiae without marked rings and with several transverse rows of long setae distally. Abdomen in some species with numerous, very long setae. Abdominal ctenidium either normally developed, consisting of specialized spines as in *Nycteribia* (*dufourii* group) or consisting of a more or less reduced row of ordinary short spines. Postspiracular sclerite absent. Male genitalia of *Nycteribia* type. Two genital plates in the female of which the dorsal may or may not bear hairs. Ventral plate with convex posterior surface.

### 12. *Penicillidia dufourii* Westwood, 1835

Description. Length 3·5–4 mm. Colour dark brown.

*Head* (Fig. 1*c*) rounded posteriorly. Eyes single, unpigmented, small. 5 or 6 rows of long, strong setae between the eyes and a row of shorter and one longer setae at the anterior ventral margin of the head. A few short spines in the middle of this row, which is continuous with the group of setae between the eyes. Palps thick, with several rows of long and short setae (Fig. 2*b*). Labella of proboscis nearly as long as theca.

*Thorax* (Fig. 70) wider than long. Length 1·5 mm. Width 2 mm. Dorsal pattern: mesonotum wide. Notopleural sutures converging posteriorly. Lateral plates of notopleural sutures narrow. Mesopleural sutures narrow, long and well sclerotized. The row of 4 to 8 notopleural hairs begins at the point where the notopleural suture branches and runs parallel to the external branch. There is sometimes a gap in the middle of the row. Median sternal suture narrow, sometimes widened for a short distance. Thoracic ctenidium consisting of about 18 pointed spines. Tibiae scalpel-shaped, slightly shorter than the femora with 3 to 4 rows of long setae distally (Fig. 4*c*).

*Abdomen female.* Tergite 1 much wider than long, with rounded sides and a shallow indentation in the middle of the posterior margin. A row of short setae along the margin of the rounded sides, which is broadly interrupted in the middle of the hind margin. Two groups of small hairs in the middle of the surface. Tergite 2

about 3 times as broad as long; roughly trapezoid and slightly protracted in the middle posteriorly; quite bare and incompletely divided along its median line. Two groups of 3 to 9 short spines near the middle of the hind margin, leaving a gap between them. Two pigmented stripes run from the two groups of spines to the anterior margin of the tergite, reaching it in the middle of each half. Tergites 3 to 6 fused into a membrane. Tergite 4 can be made out by the arrangement of the setae. It is covered with 3 or 4 rows of long setae, those of the posterior row being the longest. Behind this row the middle of the abdomen is completely bare. In the anterior lateral corners of the bare area there are two chitinous processes which project backwards. They have a sharp, darkly pigmented posterior edge and a concave posterior surface. The sides of the abdomen are covered with short hairs. Anal segment bare except for a double row of long setae at the posterior margin and an irregular row of short setae before them.

Sternite 1+2 nearly rectangular, rounded anteriorly, narrower than the abdomen, with a ctenidium of 40 to 44 short, flattened spines which stand close together. In front of them a row of long setae which may be double at the sides. The surface of the sternite is covered with short hairs which become gradually longer posteriorly. Sternites 3 to 4 membranous, covered with short hairs and groups of longer hairs at the lateral angles. Sternites 5 and 6 divided into elliptical lateral plates. The sides of sternites 5 and 6 are prolonged into lobes which bear dense groups of setae. Sternite 5 has the form of a shallow arc and sternite 6 is so strongly curved that its lateral lobes point backwards. Sternite 7 is triangular and similarly prolonged into two anal lobes with dense groups of setae. The dorsal genital plate is small, triangular, with the apex anteriorly. Its rounded posterior margin bears 5 to 7 equally spaced setae. The ventral genital plate is narrow with a rounded anterior margin and a convex posterior surface (Figs. 10*b*, 71*a*).

*Abdomen male.* Tergite 1 similar to that of female, but smaller. The indentation of the posterior margin is shallow, the gap in the middle of the posterior row of setae is smaller and the setae are longer and more widely spaced. Tergites 2 to 4 rather broad and slightly curved, densely covered with short hairs and with a row of longer setae at the posterior margin. Those of tergite 4 longer than those of tergites 2 and 3. Tergites 5 and 6 bare on the surface and with a double or triple row of very long and strong setae at the posterior margin. The long setae of tergites 4 to 6 form a dense brush. Anal segment wide and rounded, bare dorsally and covered with long hairs at the sides. A number of very long, curved setae at the posterior margin.

Sternite 1+2 similar to that of female. Sternites 3 and 4 bare in middle and covered with hairs laterally. A row of fine, not very long hairs at the posterior margin. These hairs are shorter in the middle and longer at the sides. Sternite 5 more strongly arched. Its lateral ends prolonged into broad processes with a flat surface which is densely covered with short, thick spines. In the middle of the posterior margin of the sternite a roughly triangular group of similar spines with a base of 8 to 12 spines and the apex anteriorly. There is a gap between the median group and the spines on the lateral processes. Anterior to this group of spines and on the outer side of the lateral processes there are rows of long setae (Fig. 72).

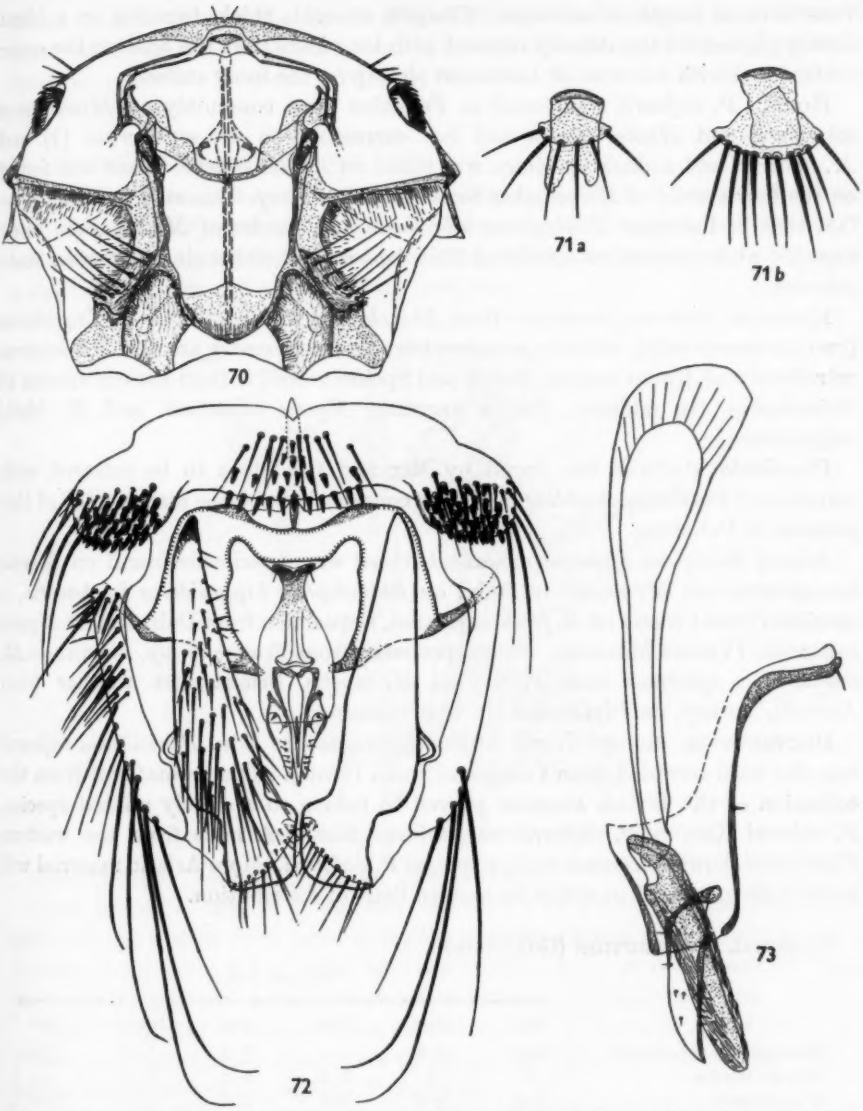



Fig. 70. *Penicillidia dufourii*. Thorax dorsal. Fig. 71. Genital plates of female of: *a*, *P. dufourii*; *b*, *P. tainani*. Figs. 72, 73. *P. dufourii*. 72, male, sternite 5 and genital area; 73, male genitalia, profile.

*Genitalia* (Figs. 72, 73). Basal arc nearly square, with broad lateral extensions. Dorsal hinge on phallobase narrow, triangular with two hairs at the basal edge. Aedeagus straight with rounded tip and without teeth at the lower surface. Apodeme about twice as long as the aedeagus. Parameres narrow, triangular, pointed distally with a straight dorsal edge which bears 3 to 4 small hairs in the distal half and a few small hairs at the sides near the lower edge. Parameres about

two-thirds of length of aedeagus. Claspers straight, thick, tapering to a blunt darkly pigmented tip, densely covered with long hairs near the base on the outer surface and with a few small hairs near the tip on the inner surface.

HOSTS. *P. dufourii* was found in Palestine most commonly on *Miniopterus schreibersi* and *Myotis myotis* and less commonly on *M. mystacinus* (?) and *M. nattereri* and a single specimen was found on *Asellia tridens*. None was found on the four species of *Rhinolophus* found in the country. This may be due to the fact that in Palestine *Miniopterus schreibersi* and species of *Myotis* often roost together while the various species of *Rhinolophus* roost either singly or in separate colonies.

Karaman, however, records it from *Rhinolophus ferrumequinum* in Yugoslavia (two specimens only), while its common hosts in that country are also *Miniopterus schreibersi* and *Myotis myotis*. Falcoz and Speiser record it from various species of *Rhinolophus*. In addition, Falcoz mentions *Myotis capaccinii* and *M. blythi oxygnathus*.

*Penicillidia dufourii* was found by Mer and Goldblum to be infected with a species of *Polychromatophilus*, and they consider it to be the main vector of this parasite in Palestine.

Among European material examined, there were specimens found on *Myotis emarginatus* and *M. myotis* in Italy, on *Rhinolophus hipposideros* in Austria, a specimen from Croatia on *R. ferrumequinum*, a specimen from Dalmatia on *Myotis capaccinii* (Vienna Museum). Some specimens from France (*Vesp. murinus* = *M. myotis* ?), a specimen from Turkey on *M. myotis*, Brussa, and another from Antioch, Turkey, on *Pipistrellus* sp. were examined.

DISTRIBUTION. Europe, North Africa, eastwards into Asia. *Penicillidia dufourii* has also been recorded from China and India (Scott). Chinese material from the collection of the British Museum proved to belong to a closely related species, *P. tainani* Kar. (= *P. dufourii* var. *tainani* Kar.). A male from the western Himalayas, however, seems to be a typical *P. dufourii*. More Asiatic material will have to be examined to define its eastern limit of distribution.

SEASONAL DISTRIBUTION (Goldblum).

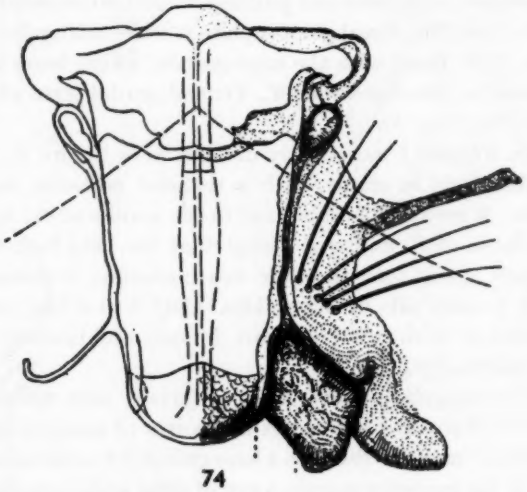
| | No. per bat | | | | | |
|---|---|---|---|---|---|---|
| | May | June | July | Aug. | Sept. | Oct. |
| *Miniopterus schreibersi* | 0–1 | 2–3 | — | 2–5 | 1–2 | Few |
| *Myotis myotis* | 0 | 0 | 0–1 | 1–2 | 0–1 | — |
| *M. nattereri* | 0–1 | 0–1 | 0–1 | 1–2 | — | — |

*P. dufourii* is closely related to two other species, *P. monoceros* Speiser and *P. tainani* Kar. *P. monoceros* is a rare species recorded only from Scandinavia (Ryberg) and Germany (Speiser). This species is characterized by the prolongation of the front into a long horn. In addition, there are differences in the thoracic pattern and the male genitalia. *P. tainani* was considered as typical *P. dufourii* by Scott. Karaman (1939) considered it as a subspecies of *P. dufourii*. Examination of material of *P. tainani* showed, however, that it differs constantly from *P. dufourii* in a number of characters. The thoracic pattern is different and the

dorsal genital plate of the female (Fig. 71*b*) is much wider and bears 10 to 12 closely standing setae as against 4 to 6 in *P. dufourii*. There are also differences in the male genitalia. It seems advisable, therefore, to consider all three forms as separate species.

13. *Penicillidia conspicua* Speiser, 1900

This species was described and illustrated by Kolenati as *P. westwoodi* and has been renamed by Speiser as this name was preoccupied. There is a short description and a rather diagrammatic figure in Falcoz (1926), and Karaman (1936) gives a short description and a figure of the genitalia.



74

Fig. 74. *Penicillidia conspicua*. Dorsal pattern of thorax.

Description. Length 3·5–4 mm. Colour brown.

*Head* similar to that of *P. dufourii*, but only 4 to 5 rows of setae between the eyes and the row of short setae along the anterior ventral margin begins a short distance from the group of setae between the eyes in most specimens. There are no spines in this row. Labella of proboscis slightly shorter than theca.

*Thorax* (Fig. 74). Dorsal pattern similar to that of *P. dufourii*, but the lateral plates of the notopleural sutures are wider and the row of 3 to 6 notopleural hairs begins further anteriorly than in *P. dufourii*.

*Abdomen female*. Tergite 1 narrow, triangular, with apex posteriorly, with rounded sides, wedged into the large rounded tergite 2 and bearing 1 to 6 short spines at its posterior tip. A group of minute hairs along its middle line. Tergite 2 large, rounded, wider than long, incompletely divided in middle. It bears a row of 15 to 20 very long, closely standing setae in the middle of its posterior margin, and lateral to them, shorter, more widely spaced setae. Its whole surface is covered with short hairs except at the posterior corners. Tergites 3 to 6 fused into a completely bare membrane. Anal segment conical with truncated end and bearing dorsally and laterally scattered short spines and a row of long setae posteriorly.

Sternite 1+2 with rounded sides and with a reduced ctenidium of 30 to 35 ordinary spines, spaced more widely than in *P. dufourii*. The median spines of the ctenidium are smaller than the lateral spines and there is sometimes a gap in the middle. In front of the ctenidium a row of about 10 long setae. Surface covered with small hairs except for a stripe along the anterior margin and in some specimens along the median line. Sternites 3 and 4 indistinct, covered with short hairs and with a few long setae laterally. Sternites 5 and 6 narrow, curved, divided into elliptical halves. The sides of the sternites are prolonged into long lobes pointing posteriorly. Both sternites are bare anteriorly and have a double row of setae at the posterior margin. Thick brushes of long setae at the tip of the lateral lobes. Anal segment covered with hairs and prolonged into two rounded lobes which also bear a brush of setae. The dorsal genital plate is wide, triangular, with a rounded anterior margin. It is fused with the anal sclerite, which bears a row of 10 to 14 long setae at the edge, forming a wide V. Ventral genital plate wide, with rounded anterior margin (Fig. 75*a*, *b*).

*Abdomen male.* Tergite 1 indistinctly divided from tergite 2. It is wider than long, roughly rhomboid in shape, with a rounded posterior angle which bears a few small hairs. A group of minute hairs in the middle of the surface. Tergites 2 to 6 similar to those of *P. dufourii*, except that the very long setae are present already on tergite 3 and that they are less numerous, standing as a whole in groups of 3 to 5 to each side of the middle. Only 2 to 4 long setae on tergite 6. Anal segment conical, with scattered hairs dorsally and laterally and a few short and long setae posteriorly.

Sternite 1+2 rectangular, shorter than in female, with rather longer hairs at the sides. Sternite 3 covered with hairs and a row of moderately long and short setae at the posterior margin. Sternite 4 bare except for some hairs at the posterior lateral angles. At the posterior margin a row of setae which are shorter and thinner in the middle and longer and stronger laterally. Sternite 5 triangular, with the apex posteriorly, nearly bare, except for 3 to 4 rows of setae at the posterior sides. These bear, in addition, one or two rows of about fifteen strong, short spines, which become gradually longer laterally, leaving the tip of the triangle bare. At the lateral ends of these rows some long setae (Fig. 76).

*Genitalia* (Figs. 76, 77). Basal arc triangular with long anterior process and small lateral flanges at the posterior ends. Dorsal hinge of phallobase broad, with 2 hairs at the base. Pigmentation branching into 2 stripes posteriorly. Aedeagus conical, 3 times as broad at the base as at the tip, with small backward-pointing teeth at the lower surface, slightly curved upwards with a blunt dorsal tooth at the tip. Apodeme nearly twice as long as aedeagus. Paramere narrow, triangular, with convex lower and concave upper margin, ending in a sharp point and bearing a row of about 10 hairs at the upper margin near the tip and a few hairs along the lower margin. They are about two-thirds of the length of the aedeagus. Claspers rather slender, curved, with darkly pigmented tip. On the outer convex side an irregular double row of long setae from the base to a short distance before the tip. On the inner surface a row of shorter setae from the tip to the beginning of the row of long setae.

HOSTS. *P. conspicua* was found in Palestine mainly on *Miniopterus schreibersi* and rarely on *Myotis myotis* and *M. mystacinus* (?). None was found on the various species of *Rhinolophus*, but Speiser and Falcoz record it from *R. euryale* and

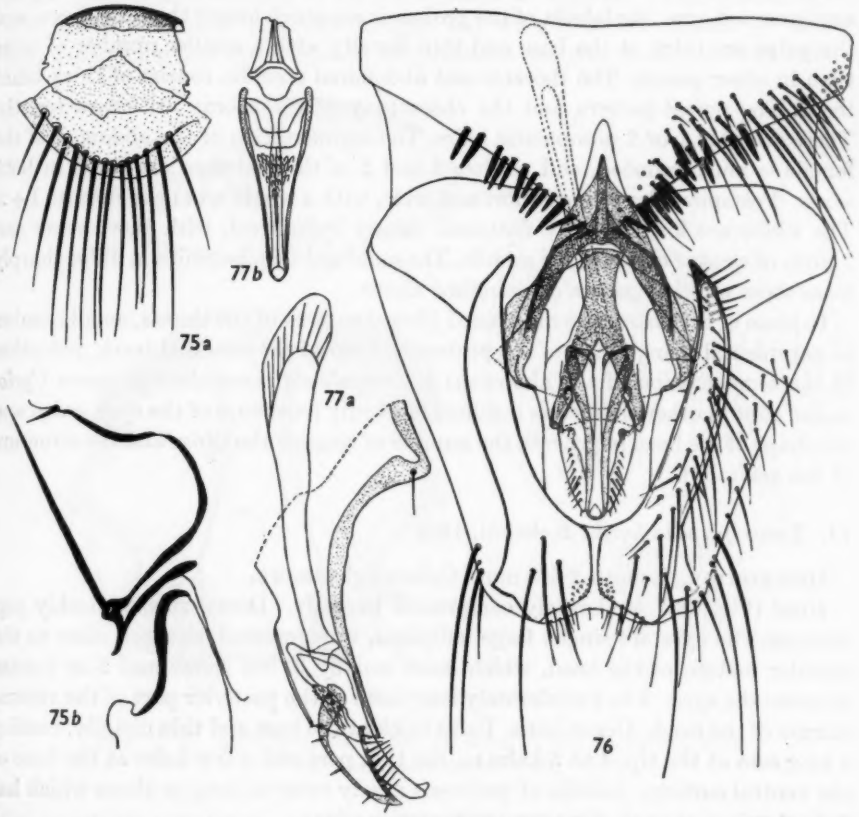



Fig. 75. *Penicillidia conspicua*. Genital plates of female: *a*, dorsal; *b*, sagittal section through genital area. Figs. 76, 77. *P. conspicua*. 76, male, sternite 5 and genital area; 77, male genitalia: *a*, profile, *b*; aedeagus dorsal.

*R. blasii* in addition to the species mentioned above, and Karaman from *R. hipposideros*. In addition to Palestinian material, specimens from Italy, France, the Balkans, and Antioch, Turkey (*Pipistrellus* sp.) were examined which did not differ from our material.

Southern Europe, from Spain to southern Russia, Armenia, Tunisia (Karaman).

SEASONAL DISTRIBUTION.

| | No. per bat | | | | | |
|---|---|---|---|---|---|---|
| | April | May | June | July | Aug. | Sept. |
| *Miniopterus schreibersi* | 0–1 | 0–1 | 1–2 | — | 5–12 | 2–5 |
| *Myotis myotis* | 0 | 0 | 0 | 0–2 | 0–2 | 0–2 |

From October to March only a few specimens were found.

## Genus **Eucampsipoda** Kolenati, 1857

Type species: *Eucampsipoda hyrtlii* Kolenati, 1856.

This genus is well defined by a number of characters. There are single, large, unpigmented eyes, the labella of the proboscis are much longer than the theca, and the palps are thick at the base and thin distally with a smaller number of setae than in other genera. The thoracic and abdominal ctenidia consist of thick blunt teeth. The dorsal pattern and the chaetotaxy of the thorax are characteristic. There are only 1 or 2 notopleural hairs. The segmentation of the abdomen of the female is much reduced, and tergites 1 and 2 of the abdomen are fused in both sexes. Postspiracular sclerite short and wide, with a single seta near sternite 1+2. The tibiae are long, slightly flattened, nearly cylindrical, with short setae and 2 rings of weaker chitin in the middle. The male and female genitalia differ sharply from those of other genera as described above.

In some of the characters mentioned (dorsal pattern of the thorax, small number of notopleural hairs, shape of the proboscis, form of the ctenidial teeth, reduction of the segmentation of the abdomen) *Eucampsipoda* resembles the genus *Cyclopodia*. But in other characters it differs markedly (structure of the eyes, palps and the shape of the head in general, the number of rings on the tibiae and the structure of the genitalia).

### 14. *Eucampsipoda hyrtlii* Kolenati, 1856

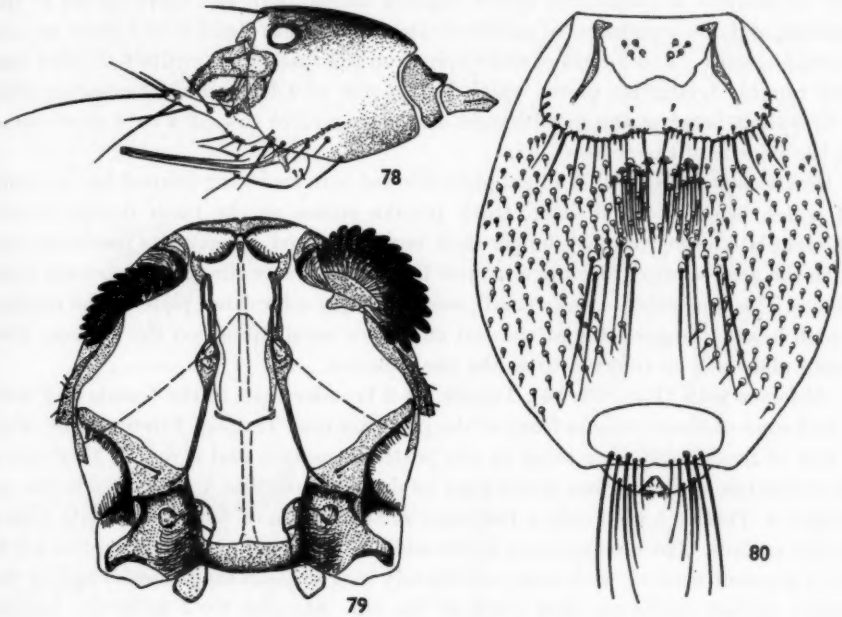
DESCRIPTION. Length 2·5–3 mm. Colour light brown.

*Head* (Fig. 78). Moderately compressed laterally. Dorsal surface darkly pigmented. The eyes are single, large, elliptical, unpigmented, situated close to the anterior margin of the head, which bears usually 2, but sometimes 3 or 4 setae between the eyes. 4 to 5 moderately long hairs at the posterior part of the ventral margin of the head. Genae bare. Palps thick at the base and thin distally, bearing a long seta at the tip, 4 to 5 hairs on the thin part and a few hairs at the base of the ventral surface. Labella of proboscis nearly twice as long as theca which has 3 short spines at each side near the posterior edge.

*Thorax* (Fig. 79). Roughly pentagonal, anterior edge between the ctenidia less than half the width of the thorax. Median sternal suture narrow, well marked, except for a short indistinct part in the middle. Oblique sutures well marked, slightly curved, joining median suture at right angles. The hairs in front of them much smaller than those behind them, except for a row of longer hairs along the base of the ctenidia. These have 16 to 18 large, curved, blunt teeth with secondary ridges. Dorsal pattern: notopleural sutures close together anteriorly, diverging posteriorly. Anterior spiracles elliptical, situated at anterior third of thorax. Mesopleural sutures well marked, broad and tapering laterally, originating at posterior third of thorax. Lateral plates of notopleural sutures absent in front of mesopleural suture. Only one long and one minute notopleural hair present. Tibiae long and cylindrical, slightly flattened, as long as femur in the fore leg or slightly shorter than femur in middle and hind leg. Two rings of weaker chitin in middle of tibiae. Setae on tibiae short, except for 3 long setae on the outer

surface. A group of minute spines apically to basal large seta on the fore tibia, and apically to the two large apical setae on the hind tibia (Fig. 4*d*).

*Abdomen female* (Figs. 80, 81). Broadly rounded or truncated in shape. First tergal plate (which consists of the fused first two tergites), roughly rectangular, with slightly concave posterior margin and with rounded corners. A row of moderately long and short setae at the posterior margin. A row of short hairs in




Figs. 78–80. *Eucampsipoda hyrtlii*. 78, head, profile; 79, thorax, dorsal; 80, female, abdomen, dorsal.

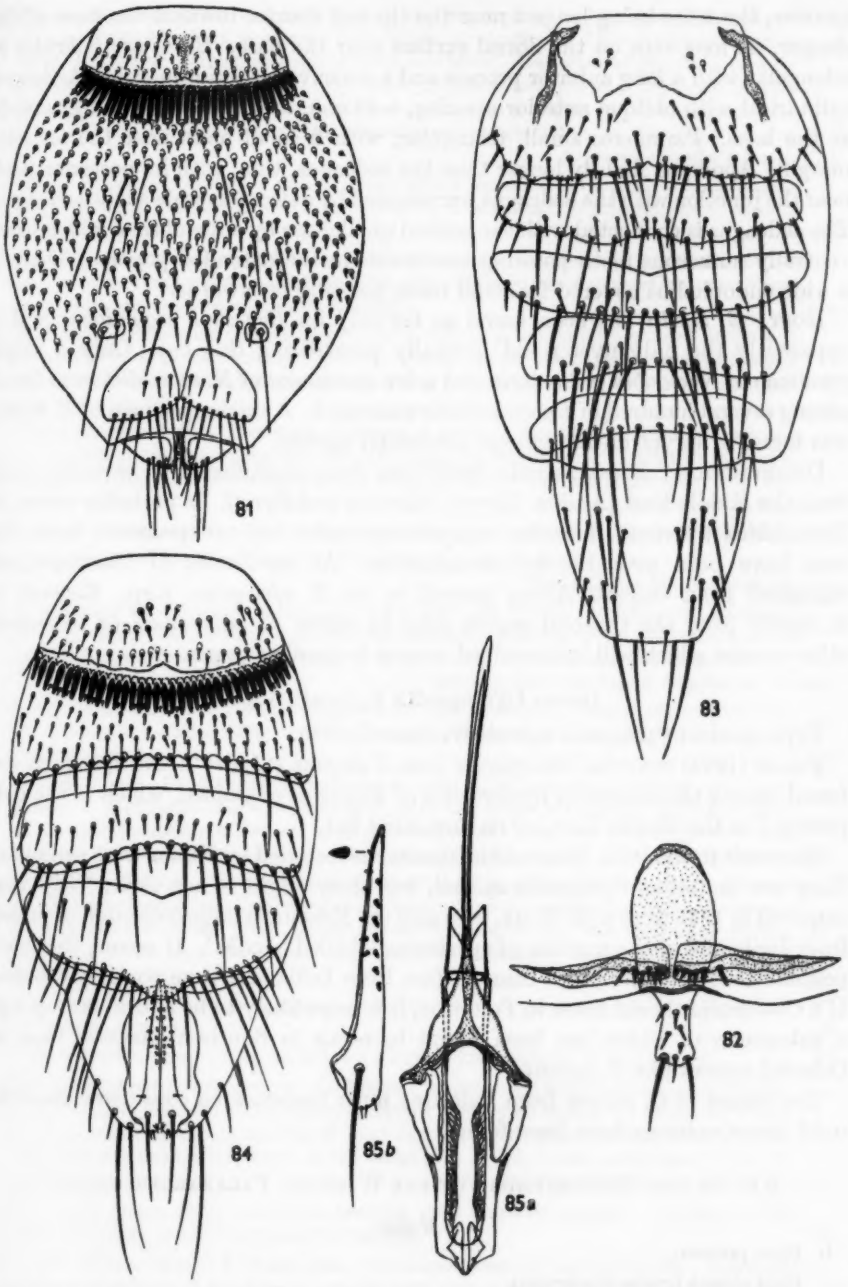
front of the marginal row and at the sides. Two groups of 3 to 4 minute hairs near the anterior border. Tergites 3 to 5 fused into a membrane without segmentation. In the middle of the area two groups of 3 to 6 long setae and in front of them, behind the middle of the first tergite, two other groups of about 10 shorter and thinner setae each. The whole area is covered with small spines, except the space between the long setae and behind them. The arrangement and number of the long setae on the abdomen has proved an important character for the differentiation of the various species of the genus. Tergite 6 broadly elliptical, bare, with a row of 8 to 10 long setae posteriorly, sometimes with a gap in the middle of the row. The median setae are shorter than the lateral setae. Anal segment very short with several setae at the tip. Sternite 1 + 2 rectangular, very short, with a ctenidium of 32 to 36 thick spines and 3 to 4 rows of short setae in its posterior half. Post-spiracular sclerite with a single moderately long seta near sternite 1 + 2 (Fig. 5*e*). Abdomen behind the basal sclerite covered with small spines. 4 long setae in the middle of sternite 3, 2 anteriorly and 2 further posteriorly and further apart.

Sternite 3 is covered with about 7 rows of short spines. The hind margin of the sternite is indicated by 2 long setae at each side and the other setae of the row are only slightly longer than the short spines of the surface. There is a narrow bare space between sternites 3 and 4 in extended specimens. The posterior row of sternite 4 consists of short spines, of which only two in the middle are slightly longer than the others. 2 to 3 rows of short spines on the surface. The posterior row of sternite 5 consists of spines slightly longer than the short spines of the surface, and of 4 long setae, of which the two lateral ones stand with a short seta on a small sclerite. 2 to 3 rows of short spines on the surface. Sternite 6 divided into two roughly triangular plates which bear a row of 4 long setae alternating with 4 to 5 short setae at the hind margin and a transverse row of 3 to 4 short setae in the middle of the surface.

*Genital plates* (Fig. 82). Ventral plate divided into two wing-shaped halves, each of which bears about 6 short, thick peglike spines at the inner dorsal corner. Dorsal plate large, rounded, longer than broad, vaulted, fused at its posterior edge with the anal sclerite, forming a narrow lip, which covers the genital opening from above. This anal sclerite has a small, more strongly sclerotized plate in the middle, which bears 2 longer setae at the end and a few small spines on the surface. This small plate will be referred to as the anal sclerite.

*Abdomen male* (Figs. 83, 84). Tergite 1+2 broader than in the female and with 2 to 3 rows of short setae in front of the posterior row. Tergites 3 to 6 narrow, with a row of moderately long setae at the posterior margin and a row of short hairs in the middle of the surface which may be double on tergite 3, and a single row on tergite 4. Tergite 5 with only a few hairs in the middle or bare and tergite 6 bare on the surface. 2 to 4 longer setae in the middle of the hind margin of tergites 4 to 6. Anal segment conical, with some moderately long setae in the posterior half of the dorsal surface and some long setae at the tip. Sternite 1+2 as in the female. Sternite 3 narrow with rather short setae posteriorly and 2 to 3 rows of short setae on the surface. Sternite 4 similar, but some of the posterior setae longer, and only 1 row of short setae on the surface which may have a gap in the middle in some specimens. The anterior margin of sternite 4 is straight or slightly convex. Sternite 5 is longer and triangularly produced in the middle of the hind margin. A row of long and short setae at the sides of the hind margin. There are a few short, thin hairs at the process in the middle and in most specimens 2 short, thick, and 2 much smaller pegs. In some specimens these pegs are absent. Surface of the sternite bare except for a transverse row of short setae which is broadly interrupted in the middle. Anterior margin indented in the middle.

*Genitalia* (Figs. 7, 85). The structure of the genitalia has been described in detail (pp. 171 & 172). Claspers long (0·43 mm.), straight, and pointed in dorsal view. In profile, the tip of the clasper is widened and has a short tooth close to the tip. On the dorsal surface 2 rows of 6 to 8 short, thick pegs from near the tip to about the middle of the claspers. The lateral row begins nearer the tip and the dorsal row reaches further basally. There is sometimes a short additional row between the two others. A few minute spines anteriorly to the dorsal row of pegs. A row of thin hairs at the lateral border of the ventral surface from near the tip to the basal




Figs. 81–85. *Eucampsipoda hyrtlii*. 81, female, abdomen, ventral; 82, female, genital plates; 83, male, abdomen, dorsal; 84, male, abdomen, ventral; 85, male: *a*, genitalia ventral; *b*, clasper.

quarter, the hairs being longest near the tip and shorter towards the base of the clasper. A long seta on the dorsal surface near the base. Basal arc (sternite 9) triangular with a long anterior process and a concave posterior margin. Aedeagus cylindrical with oblique anterior opening, 0·43 mm. long, 8 times as long as wide at the base. Parameres small, triangular, with 2 small hairs near the anterior margin. Apodeme slightly longer than the aedeagus, thin, with its posterior part, near the junction with the aedeagus, surrounded by a sleeve as wide as the aedeagus. The aedeagus is connected with the conical phallobase by a membrane which bears ventrally numerous teeth pointing backwards in the extended position. There is a wide endophallus covered by small teeth pointing backwards.

HOST. *E. hyrtlii* has been found so far only on *Rousettus aegyptiacus* and is apparently the only nycteribiid normally parasitizing this bat. Only a single specimen of *Nycteribia pedicularia* and a few specimens of *N. schmidlii* were found among several hundred of *Eucampsipoda* examined. A single specimen of *E. hyrtlii* was found in Egypt on *Rhinopoma hardwickei cystops*.

DISTRIBUTION. *Eucampsipoda hyrtlii* has been identified with certainty only from the Middle East (Arabia, Egypt, Palestine and Syria). It probably occurs in East Africa wherever *Rousettus aegyptiacus* occurs, but no specimens from this area have been available for examination. All specimens of *Eucampsipoda* examined from tropical Africa proved to be *E. africanum* n.sp. Records of *E. hyrtlii* from the Oriental region refer to either *E. latisternum* or to several other species which will be described as new in another paper.

## Genus **Cyclopodia** Kolenati, 1863

Type species: *Cyclopodia sykesii* Westwood, 1835.

Falcoz (1924) recorded this species from Palestine ('Judée'). No specimen was found among the numerous nycteribiids of *Rousettus* examined, which is the only pteropid in the Middle East, or on any other bat.

On a visit to the Paris Museum the specimens recorded by Falcoz were examined. They are doubtless *Cyclopodia sykesii*, but their origin is not clear. They were acquired in 1836 from a Mr Roux, who sold the Museum a large collection of insects from India and also a number of specimens labelled 'Judée'. It seems, therefore, possible that the *Cyclopodia* came in fact from India and were wrongly labelled. If a *Cyclopodia* should occur in Palestine, it is more likely to be a form of *C. greeffi*, a subspecies of which has been found to occur in Southern Arabia, than an Oriental species like *C. sykesii*.

The record of *C. sykesii* from Palestine must therefore be considered doubtful until new specimens have been found.

### KEY TO THE NYCTERIBIIDAE OF THE WESTERN PALAEARCTIC REGION

*Males*

1. Eyes present. 2
— Eyes absent (genus *Nycteribia*). 8
2. Tibiae without well-marked rings and with several rows of long setae distally. Labella of proboscis as long as theca or shorter. Spines of thoracic ctenidium narrow and pointed. 3

2. Tibiae long and cylindrical, with 2 rings and short setae in the middle. Labella of proboscis twice as long as theca. Eyes single, unpigmented. Head pigmented dorsally. Spines of thoracic ctenidium thick and blunt. Notopleural sutures diverging posteriorly, mesopleural suture originating in posterior third of notopleural suture. One large and one minute notopleural hair. Claspers straight, pointed and with two rows of short, peg-like spines. On *Rousettus aegyptiacus* (Figs. 78, 79, 85).
**Eucampsipoda hyrtlii** Kol.

3. Large setose insects (3·5–4 mm.). Head broad, rounded posteriorly. Eyes single, unpigmented. Haltere groove cover present. Postspiracular sclerite absent (genus *Penicillidia*). 4

— Smaller, less setose insects (2–3 mm.). Head laterally compressed. Eyes with double lenses on common pigmented base (Fig. 52). Haltere groove cover absent. Postspiracular sclerite present (genus *Basilia*). 6

4. Abdominal ctenidium consisting of closely standing, specialized spines. Sternite 5 of abdomen with two lateral, broad processes which are covered with numerous short, thick spines and with a median group of similar spines. Claspers thick and straight (Fig. 72). 5

— Abdominal ctenidium consisting of ordinary, widely spaced spines. Sternite 5 without lateral processes, triangular with the apex posteriorly and rows of short spines along the posterior sides of the triangle. Claspers slender and slightly curved (Fig. 76).
**Penicillidia conspicua** Speiser

5. Anterior dorsal margin of head slightly convex. **Penicillidia dufourii** Westwood

— Anterior dorsal margin of head prolonged into a long horn.
**Penicillidia monoceros** Speiser

6. Tibiae slender, 4·5 times as long as wide. Sternite 5 with a double row of about 18 short spines in the middle of the hind margin. All tergites of abdomen bare in middle. Aedeagus curved, tapering to a point. On *Pipistrellus kuhli* (Figs. 65–67).
**Basilia bathybothyra daganiae** n.subsp.

— Tibiae shorter, about 3 times as long as wide. Tergites 2 to 4 of abdomen covered with short hairs in middle. Sternite 5 with single row of 6 to 8 short spines in middle of hind margin. 7

7. Length 3 mm. Ocular sclerite cylindrical with wide posterior opening. A row of about 15 notopleural hairs beginning close to the anterior spiracle. Aedeagus with long point, consisting of several long, thin teeth and dorsal membrane covered with teeth. Parameres with smooth ventral edge (Figs. 53–55). **Basilia nattereri** Kol.

— Length 1·8–2·2 mm. Ocular sclerite spherical with small posterior opening. A row of 9 to 12 notopleural hairs beginning at a distance from the anterior spiracle. Aedeagus with broadly rounded point which curves backwards and has serrations on the outer side. No teeth on dorsal membrane of aedeagus. Parameres with strong ventral tooth (Figs. 58, 61). **Basilia nana** n.sp.

8. Tibiae laterally compressed, very broad, 2·25 to 2·4 times as long as wide (subgenus *Nycteribia*) (Fig. 4*b*). 9

— Tibiae slender, scalpel shaped, 3·5–4·5 times as long as wide (Fig. 4*a*). 11

9. Mesonotal space rather narrow. Abdomen long and narrow with long, parallel-sided anal segment. Sternite 5 long, strongly convex posteriorly with a row of about 14 very short, peg-like spines in middle of hind margin. Aedeagus tapering with a backwards bent, bifid tip and teeth in the basal part of the dorsal membrane (Figs. 27, 32, 33). **Nycteribia (Nyct.) schmidlii** Schiner

— Mesonotal space wider, abdomen broader, anal segment conical. Sternite 5 with nearly straight or concave posterior margin. 10

10. Length 3 mm. Tergite 4 of abdomen with large group of hairs in middle. Posterior margin of sternite 5 concave in middle and with a row of 14 to 18 short spines. Phallobase conical, aedeagus conical, tapering to a blunt tip which has a short ventral tooth. Parameres with pointed tip (Figs. 18–21).
**Nycteribia (Nyct.) latreillii** Leach

10. Length 2·5 mm. Tergite 4 bare or with only a few hairs in middle. Sternite 5 with a row of 9 to 12 short spines in middle of hind margin. Phallobase with marked distal bulge. Aedeagus rather wide and short, with blunt tip and a subapical tooth rather far from tip. Parameres with blunt tip (Figs. 13–15).
**Nycteribia (Nyct.) pedicularia** Latr.

— Length 2–2·25 mm. Tergite 4 as in *N. pedicularia*. Sternite 5 with a row of 7 to 8 short spines in middle of hind margin. Phallobase conical, without distal bulge. Aedeagus slender with subapical tooth closer to tip (Figs. 24–26).
**Nycteribia (Nyct.) kolenatii** n.nom.

11. Tibiae 3·5 times as long as wide. Sternite 5 with concave posterior margin and a single row of 7 to 8 short spines. Anal segment conical. Aedeagus weakly sclerotized with broadly rounded tip. Parameres narrow, triangular (Figs. 36, 37).
**Nycteribia (Acrocholidia) vexata** Westwood

— Tibiae 4·5 to 5 times as long as wide and with a group of small spines near base of fore tibia. Sternite 5 more or less convex posteriorly or bilobed with several rows of spines in middle. Parameres with beak-like process, sometimes fused with phallobase and with several long hairs. Aedeagus curved, tapering to a rounded point (Figs. 40, 41) (subgenus *Stylidia*). 12

12. Posterior margin of sternite 5 markedly convex, with a group of 4 to 5 rows of spines which are longer posteriorly and shorter anteriorly. Anal segment wide and rounded. Claspers thick, straight and blunt. Two broad, bare processes in the anterior lateral corners of the anal segment ventrally (Fig. 40).
**Nycteribia (Stylidia) biarticulata** Herm.

— Length 3 mm. Colour brown. Tergites 5 and 6 divided in middle. Posterior margin of sternite 5 divided into two broad lobes, each bearing 4 to 5 rows of short spines. Anal segment conical. Basal processes absent. Claspers thick, curved and tapering to a long point. Tip of aedeagus thin with a small knob (Figs. 44–47).
**Nycteribia (Stylidia) biloba** n.sp.

— Length 2·5 mm. Colour yellowish. Tergites 5 and 6 of abdomen undivided. Posterior margin of sternite 5 whole, slightly convex, with an irregular double row of about twenty-four spines. Claspers slender, straight, tapering to a blunt point. Tip of aedeagus blunt (Figs. 48–50). **Nycteribia (Stylidia) integra** n.nom.

*Females*

1. Eyes present. 2

— Eyes absent (genus *Nycteribia*). 7

2. Tibiae without well-marked rings and with several rows of long setae distally. Labella of proboscis as long as theca or shorter. Spines of thoracic ctenidium narrow and pointed. 3

— Tibiae long, cylindrical, with two rings and short setae in middle. Labella of proboscis twice as long as theca. Eyes single, unpigmented. Head pigmented dorsally. Spines of thoracic ctenidium thick and blunt. Notopleural sutures diverging posteriorly, mesopleural suture originating in posterior third of notopleural suture. One large and one minute notopleural hair. Abdomen nearly without segmentation, membranous, covered with small spines and with two groups of long setae in middle of dorsum. Tergite 6 elliptical, with long setae at the hind margin. Ventral genital plates wing-shaped with 5 to 6 short spines. Dorsal genital plate wide, rounded (Figs. 79–82). **Eucampsipoda hyrtlii** Kol.

3. Large, setose insects (3·5–4 mm.). Head broad, rounded posteriorly. Eyes single, unpigmented. Haltere groove cover present. Postspiracular sclerite absent (genus *Penicillidia*). 4

— Smaller, less setose insects (2–3 mm.). Head laterally compressed. Eyes with double lenses on common pigmented base (Fig. 52). Haltere groove cover absent. Postspiracular sclerite present (genus *Basilia*). 5

4. Tergite 1 of abdomen pointed posteriorly with a few short spines at tip. Tergite 2 large, rounded, covered with small hairs and with a row of about 20 long setae posteriorly. Abdominal ctenidium consisting of widely spaced ordinary spines. Dorsal genital plate broad with an angular row of 10 to 14 long setae (Fig. 75).
**Penicillidia conspicua** Speiser

— Tergite 1 wide, rounded, with an indentation in the middle and a row of setae at the sides of the posterior margin. Tergite 2 broad, rectangular, bare, with a group of short spines in each half of hind margin. Two chitinous processes in posterior part of dorsum of abdomen. Abdominal ctenidium consisting of closely standing specialized spines. Dorsal genital plate small, with about 6 long setae posteriorly. Frontal margin of head slightly rounded (Fig. 71*a*). **Penicillidia dufourii** Westwood

— As *P. dufourii*, but frons prolonged into a long horn.
**Penicillidia monoceros** Speiser

5. Tibiae slender, 4·5 times as long as wide. Tergite 1 with two processes posteriorly, each bearing 3 to 5 long setae. Second tergal plate heart-shaped, incompletely divided in middle with 3 long and several short setae at posterior processes (Figs. 68, 69). **Basilia bathybothyra daganiae** n.subsp.

— Tibiae shorter, about 3 times as long as wide. Tergite 1 rounded posteriorly with a row of long setae. Second tergal plate square or rectangular with a row of long and short setae posteriorly. **6**

6. Length 3 mm. Ocular sclerite cylindrical with wide posterior opening. A row of about 15 notopleural hairs beginning close to the anterior spiracle. Median sternal suture narrow, only slightly widened in middle. Second tergal plate rectangular, much wider than long, covered with hairs except for a narrow stripe at the sides. Sternite 6 divided into 2 plates (Figs. 56, 57). **Basilia nattereri** Kol.

— Length 1·8–2·2 mm. Ocular sclerite spherical with small posterior opening. A row of 9 to 12 notopleural hairs, beginning at a distance from the anterior spiracle. Median sternal suture widened in middle half. Second tergal plate of abdomen nearly square with a diamond-shaped field of small hairs in middle. Sternite 6 divided into 3 plates (Figs. 62, 63). **Basilia nana** n.sp.

7. Tibiae laterally compressed, very broad, 2·25–2·4 times as long as wide. Hind margin of tergite 1 of abdomen with short hairs (subgenus *Nycteribia*). **8**

— Tibiae slender, 3·5 or 4·5 times as long as wide. Hind margin of tergite 1 of abdomen with longer hairs. **10**

8. Mesonotal space rather narrow. Five tergites on abdomen, tergite 3 elliptical, with 6 long setae posteriorly. Dorsal genital plate elliptical with several widely spaced short hairs. Ventral genital plate absent (Figs. 28–30).
**Nycteribia (Nyct.) schmidlii** Schin.

— Mesonotal space wider, only 4 tergites on abdomen. Tergite 3 absent. **9**

9. Length 3 mm. Tergite 2 of abdomen long, strongly convex posteriorly. Dorsal genital plate triangular with truncated sides and a row of 11 to 14 strong, short spines. Ventral genital plate wide. Sides of abdomen covered with small spines (Figs. 9*a*, 16, 17). **Nycteribia (Nyct.) latreillii** Leach

— Length 2–2·25 mm. Tergite 3 similar to that in *N. latreillii* or pointed posteriorly. Sides of abdomen covered with small spines. Dorsal genital plate triangular with 8 to 10 long, widely spaced setae. Ventral genital plate narrow (Figs. 9*c*, 22, 23).
**Nycteribia (Nyct.) kolenatii** n.nom.

— Length 2·5 mm. Tergite 2 shorter, less strongly curved posteriorly. Sides of abdomen bare. Dorsal genital plate triangular with a row of 12 short spines posteriorly. Ventral genital plate narrow (Figs. 9*b*, 11, 12).
**Nycteribia (Nyct.) pedicularia** Latr.

10. Tibiae 3·5 times as long as wide. Hind margin of tergite 1 of abdomen with a row of long setae. Tergite 2 with nearly straight posterior margin. Dorsal genital plate concave posteriorly with 4 to 6 widely spaced short spines. Ventral genital plate absent (Figs. 9*e*, 35). **Nycteribia (Acrocholidia) vexata** Westw.

10. Tibiae 4·5 or 5 times as long as wide, with a group of small spines near base of fore tibia. Second tergal plate very large. Dorsal genital plate shield-shaped. Ventral genital plate absent. 11

11. Two long thin processes with long setae at the tip on the posterior end of the abdomen. Tergite 6 large, roughly triangular. Genital plate small. (0·2 × 0·15 mm.) (Fig. 39*a*). **Nycteribia (Stylidia) biarticulata** Herm.

— No such processes. 12

12. Tergite 6 divided into two rounded plates. Genital plate large (0·35 × 0·27 mm.) (Fig. 39*b*). **Nycteribia (Stylidia) biloba** n.sp.

— Tergite 6 undivided, elliptical. Genital plate small (0·14 × 0·1 mm.) (Fig. 51). **Nycteribia (Stylidia) integra** n.nom.

*Eucampsipoda aegyptia* Macquart is not included in the key.

## IV. STREBLIDAE

This family will be dealt with much more briefly than the Nycteribiidae, as it has been worked out in great detail, mainly by Jobling, who has published a long series of papers on the family in *Parasitology* and the *Transactions of the Royal Entomological Society of London* (1929–52).

Jobling (1951) discussed the homology of the male genitalia in some genera in connexion with his study of the systematic position of the family. In the description of various species he used the relative length of the apodemes and the form of the hypopygium, but not of other parts of the genitalia.

A comparison of the genitalia of *Nycteribosca kollari* and *N. alluaudi* showed, however, that there are marked differences in the male and female genitalia which facilitate identification.

*Male genitalia.* The aedeagus of *Nycteribosca* consists of a long, membranous tube, tapering to a fine point which is curved towards the ventral side. Ventrally the wall of the tube is strengthened by a chitinous rod. The aedeagus is articulated posteriorly with a long, lightly curved, rod-like apodeme in *N. kollari*. In *N. alluaudi* the aedeagus is fused with the apodeme. The aedeagus lies within a gutter, the so-called aedeagus sheath, which lies ventral to the aedeagus. This sheath consists of the phallobase, the parameres and the 9th sternite which are all fused into a rigid structure. The aedeagus sheath is distinctly asymmetrical. The right paramere is stronger and wider, and its ending is characteristic for the species. Sternite 9 is compressed laterally and lies in a plane either at right angles or at about 60° to the plane of the parameres. The parameres are two long tapering processes with minute hairs at the tip. In *N. kollari* the right paramere is distinctly hooked at the tip, while in *N. alluaudi* both parameres are straight with a rounded tip (Fig. 86A, B). Sternite 9 (called basal arc in the Nycteribiidae in this paper) is indicated by a ventral bulge which continues basally into a long, curved apodeme. In *N. alluaudi* the phallobase and sternite 9 are much narrower and the asymmetry is less marked than in *N. kollari*. The whole genital apparatus, including the phallobase, is retracted into the abdomen in the resting position, so that only the tips of the parameres and of the aedeagus protrude from the body. Dorsal to the genitalia lies a curved or S-shaped rod which extends from the ventral side of the anus to the aedeagus sheath. The homology of this sclerite is

not clear. The digitiform processes are much thinner in *N. alluaudi* than in *N. kollari*. Ventral to the anus lie two sclerites, each of which bears 2 long setae. Their position makes it likely that they represent the rudimentary claspers.

The male genitalia of two species of *Raymondia* examined (Fig. 87) differ markedly from those of *Nycteribosca*. The aedeagus s similar to that of *Nycteribosca*, but is shorter, and in the resting position lies completely invaginated in a membranous tube which bears numerous long spines pointing backwards when the aedeagus is extended. The genitalia of *Raymondia* thus differ from those of *Nycteribosca* in a similar way as the genitalia of *Eucampsipoda* differ from those of *Nycteribia*. The left paramere is much shorter than the right paramere. The asymmetry of the genitalia of *Raymondia* is more strongly marked than in *Nycteribosca*. The curved rod which lies dorsal to the genitalia is widened posteriorly into a broad plate (Fig. 87*b*).

*Female genitalia.* In his description of the female hypopygium of *N. gigantea* Jobling mentions four sclerites in the vicinity of the genital opening as follows: 'there is an irregular, rounded plate more or less closely united with sternite 7. This plate (which Jobling interprets as sternite 8) closes the genital opening. Sternite 10 extends from below the anus to the dorsal side of the genital opening and near its ventral edge there is a small plate, movably attached to sternite 10 which probably represents sternite 9.' These sternites are apparently homologous with the sclerites called genital plates in the Nycteribiidae in this paper. In the two species examined, sternites 8 and 9 are not discernible and the genital opening apparently lies between sternite 7, the shape of which differs considerably in the various species, and sternite 10 which also has a distinctive form. Sternite 10 in *N. kollari* has two strongly sclerotized, curved horns at its ventral edge and a broad process between them, while in *N. alluaudi* the ventral edge of sternite 10 is smooth with rounded corners. Sternite 7 in *N. kollari* is a large rounded plate with a strongly convex posterior surface, covered with short setae except at its posterior margin, while sternite 7 in *N. alluaudi* is much smaller, triangular, with a nearly straight posterior margin which bears a row of 6 to 8 short setae on raised bases and two longer and a few shorter setae on its surface (Fig. 88).

## Genus **Nycteribosca** Speiser, 1899

### 1. *Nycteribosca kollari* Frauenfeld, 1855

Syn. *Nycteribosca africana* Walker, 1849 (pro parte); Jobling, 1934.

*N. kollari* was described by Frauenfeld from Egypt as a parasite of *Rhinopoma microphyllum* and *Rhinolophus clivosus*. Jobling (1934) considered it to be identical with *Nycteribosca africana* Walker which was described originally from South Africa. Comparison of material of *N. africana* from South Africa with specimens from Palestine showed, however, that there are some constant differences which seem marked enough for both forms to be considered as distinct species. It is possible, however, that these two forms represent the extremes of a long series of transitional forms. Examination of material from various localities in the Mediterranean and in tropical Africa will be necessary to decide definitely whether the

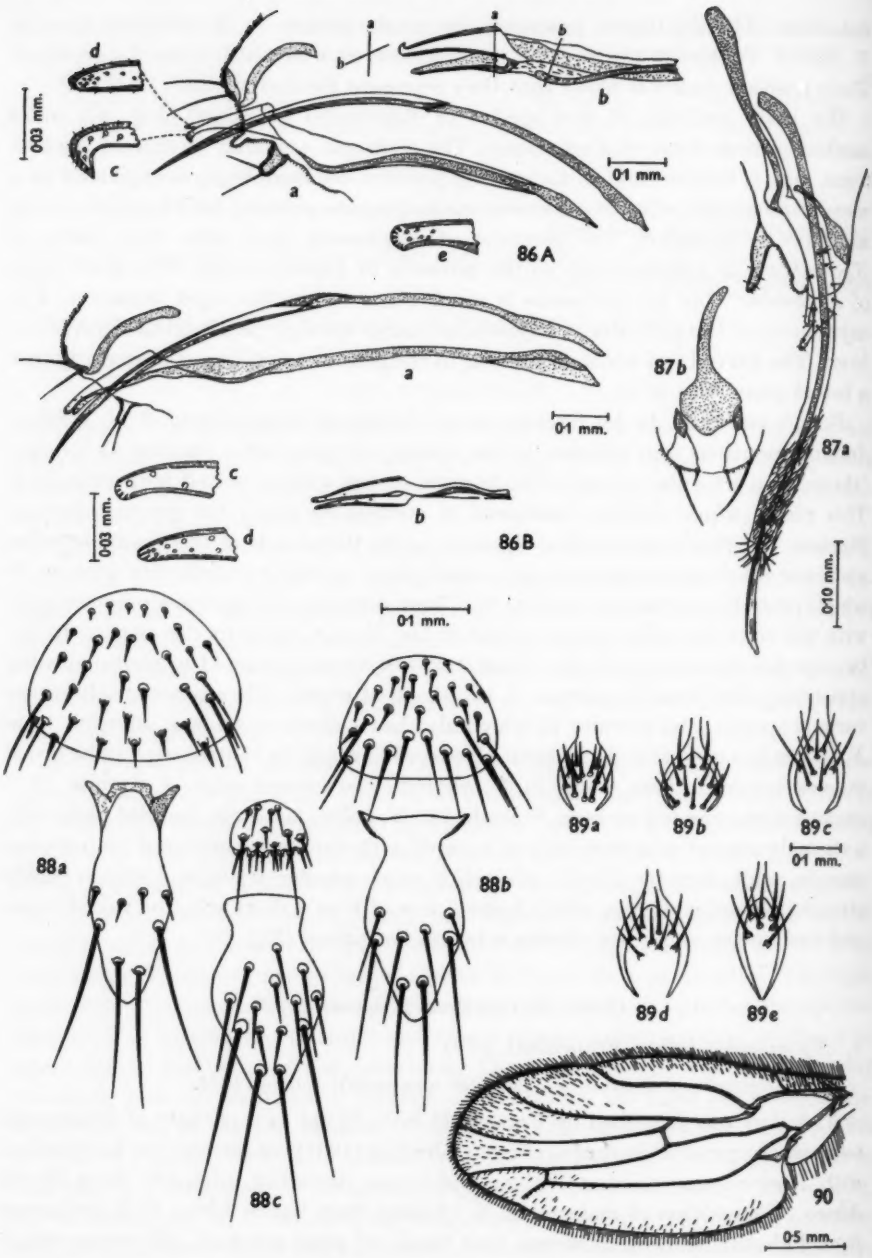



Fig. 86A. Male genitalia of *Nycteribosca kollari*: *a*, lateral view; *b*, ventral; *c*, tip of right paramere; *d*, tip of left paramere; *e*, tip of right paramere of *N. africana*. Fig. 86B. Male genitalia of *N. alluaudi*: *a*, lateral view; *b*, ventral; *c*, tip of right paramere; *d*, tip of left paramere. Fig. 87. Male genitalia of *Raymondia huberi*, extended position: *a*, lateral view; *b*, ventral view of curved rod between anus and genitalia. Fig. 88. Sternites 7 and 10 of females of: *a*, *Nycteribia kollari*; *b*, *N. africana*; *c*, *N. alluaudi*. Fig. 89. Postvertex of: *a–b*, *N. kollari*; *c*, *N. africana*; *d–e*, *N. alluaudi*. Fig. 90. Wing of *N. kollari*.

two forms are different species or belong perhaps to a single variable species with a very wide range of distribution. Provisionally, however, the name *N. kollari* will be used for the Mediterranean form and it is considered distinct from *N. africana* from South Africa.

The most important differences between the two species are as follows: the postvertex is ovoid in *N. africana* and much broader, nearly triangular in *N. kollari* (Fig. 89). The wings of both species differ only in minor details. $R_{4+5}$ and $M_{1+2}$ diverge more strongly near the wing margin in *N. kollari* than in *N. africana*. Cell $2M2$ is shorter in *N. kollari* and the distal parts of $M_{1+2}$ and $M_4+Cu_1$, forming its anterior and posterior borders, are more strongly curved in *N. kollari* than in *N. africana* (Fig. 90). Sternite 7 of *N. kollari* is large, rounded and covered with short setae while that of *N. africana* is much smaller, with long setae near the posterior margin. Sternite 10 of *N. kollari* has two strongly sclerotized horns at its ventral edge between which there is a broad process, while that of *N. africana* has broader horns without the median process (Fig. 88). In the male of *N. africana* the tip of the right paramere is much less hooked than in *N. kollari* (Fig. 86 A, *a–e*).

*N. kollari* was commonly found on the four species of *Rhinolophus* in Palestine and only a single specimen on *Miniopterus schreibersi*. Falcoz records the species also from other species of *Rhinolophus*, from *Myotis myotis* and *Asellia tridens*; and Jobling from *Rhinopoma microphyllum* and *Vespertilio murinus* L. This is the only species of Streblidae which has a wide distribution in the Mediterranean, south of the winter isotherm of + 10° C., as pointed out by Jobling. An additional record is from a cave near Antioch from *Rhinolophus* sp. exactly on the + 10° C. winter isotherm, collected by Prof. Kosswig, Istanbul.

2. *Nycteribosca alluaudi* Falcoz, 1923

Syn. *N. diversa* Frauenfeld,*

This species was found in Palestine only on *Rhinolophus microphyllum* and *R. hardwickei arabium* in a cave near Tiberias. It was found to be present in small numbers in January and more commonly in March and April. The following diagnosis gives the main characters for the differentiation of *Nycteribosca kollari* and *N. alluaudi*.

1. Postvertex nearly as long as broad, roughly triangular, covered with numerous (8–16) short setae. Theca of labium as broad as long. $R_{2+3}$ gently curved towards costa. $R_{4+5}$ and $M_{1+2}$ markedly divergent near wing margin. Alula pointed distally. Setae on thorax numerous, dense and short. Sternite 7 of female large, rounded, with many short setae except at the posterior margin. Sternite 10 with two curved, strongly sclerotized processes at the ventral edge. Right paramere of male distinctly hooked at tip. Phallobase and sternite 9 wide. Digitiform processes thick and conical. **Nycteribosca kollari**

2. Postvertex ovoid, distinctly longer than broad, with a few (3–9) setae only. Theca of labium conical, much longer than broad. $R_{2+3}$ sharply bent towards costa. $R_{4+5}$ and $M_{1+2}$ parallel near wing margin. Alula rounded distally (Fig. 91). Setae on thorax long and strong, more widely spaced than in *N. kollari*. Sternite 7 small, triangular or rounded anteriorly, with a row of short setae on raised bases at the

* Examination by Jobling of Frauenfeld's type of *Nycteribosca diversa* from the Museum in Vienna proved this to be an abnormal specimen of *N. alluaudi* with a supernumerary branch of vein $R_{2+3}$. (Personal communication.)

straight posterior margin and with 2 long and some short setae on its surface. Sternite 10 with smooth posterior edge and rounded corners. Both parameres of male slender with straight, rounded tip. Phallobase and sternite 9 narrow. Digitiform processes thin and cylindrical. **Nycteribosca alluaudi**

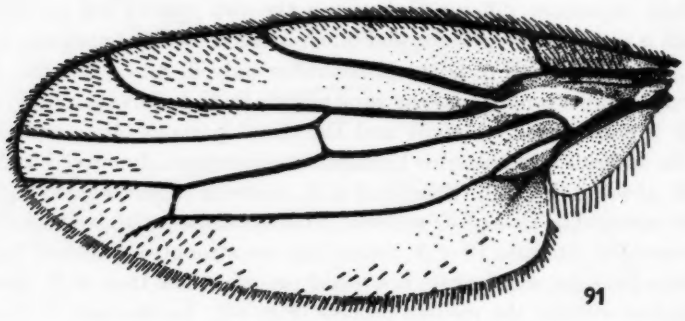



Fig. 91. Wing of *N. alluaudi* (after Jobling, 1939).

## Genus **Raymondia** Frauenfeld, 1855

*Raymondia setosa* Jobling, 1930.

A single female was found on *Asellia tridens* in Jaffa on 4 December 1949. No further specimens were found subsequently although over 50 bats were examined. This species was first considered by Jobling as a subspecies of *R. huberi* (1930) and was subsequently raised to specific rank (1939). Only the male was described by Jobling and a description of the female is therefore given.

DESCRIPTION. Length 1·7 mm. Wing length 1·5 mm.

*Head* (Fig. 92). The latero-vertices are well defined with a chitinous ridge along the anterior margin. They bear a row of 10 to 12 long and short setae along the anterior and inner margin, of which one seta in the middle of the anterior lateral part is particularly strong. This row is double towards the inner margin. There is a row of some long and short setae along the lateral margin and a very strong and a weaker seta in the middle of the posterior part of the sclerite. The setae are much more numerous and reach further posteriorly than in *R. huberi*, where the posterior part of the latero-vertices is nearly bare. The curved row of setae around the gular region is shallower than in *R. huberi* and consists of only 12 setae as against 16 in *R. huberi*. The theca of the labium is conical and its sides are less strongly curved than in *R. huberi*.

*Thorax*. The hairs on the prescutum are numerous and about twice as long as the distance between them. The hairs in the lateral part of the scutum are more numerous than in *R. huberi*. The small marginal setae of the scutellum are arranged as in *R. huberi*. Wing similar to those of *R. huberi* but there are 3 to 4 rows of small hairs in cell 1*R*, a double row in cell 3*R*, a single row in cell 5*R* and a few single hairs in cell 2*M*2. Calypteron with a strong terminal seta and a smaller seta close to it, while in *R. huberi* the terminal seta stands on a long process and the small seta stands much further basally (Fig. 93).

*Abdomen.* A row of about 10 long, spirally striated setae standing on chitinous plates behind the basal tergite at each side. Apical cone dorsally with 6 longer setae in the basal part and with about 8 short and 2 longer setae in the terminal part. On the ventral side of the apical cone several short and 2 longer setae. A small triangular plate with truncated apex and 4 setae probably represents sternite 7.

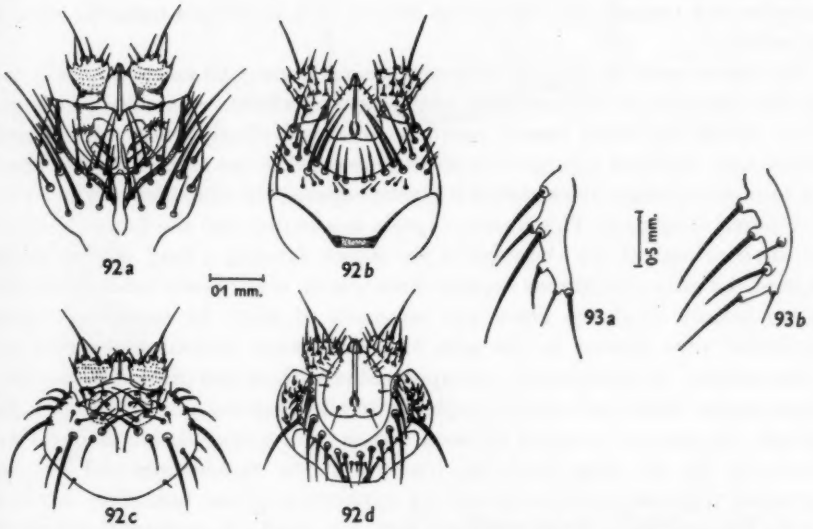



Fig. 92. Head of *Raymondia setosa*: *a*, dorsal; *b*, ventral. Head of *Raymondia huberi*: *c*, dorsal; *d*, ventral. Fig. 93. Calypteron of: *a*, *R. setosa*; *b*, *R. huberi*.

## Genus **Ascodipteron** Adensamer, 1896

*Ascodipteron rhinopomatos* Jobling, 1952.

Fifteen embedded females were found among seventy-five specimens of *Rhinopoma microphyllum* and *R. hardwickei arabium* in a cave near Tiberias on 29 March 1950. Three more were found among eleven bats on 11 April 1951, and five specimens among fourteen bats on 29 January 1952. The three females found in 1951 laid four puparia which were kept at a temperature of 27° C. One male and two females hatched after 35–37 days. One puparium did not hatch, and all three insects died 2 or 3 days after hatching. In 1952, three females were kept alive and laid three puparia, one of which was accidentally lost and the other two, a male and a female, hatched after 34 days.

Specimens were sent to Dr B. Jobling who informed us that they were *Ascodipteron rhinopomatos* which he had described as a new species from *Rhinopoma hardwickei cystops* from Egypt in 1952.

All females of *Ascodipteron rhinopomatos* were found one per bat, except in one case where there were two specimens on a bat. The position of the females on the bat is characteristic and differs from that recorded from most other species which

were found embedded near the base of the ears. A specimen of an undescribed species of *Ascodipteron* from South Africa was found embedded in the ocular cavity. Only *A. jonesi*, according to Jobling, was found on the wings and *A. phyllorhinae* on the dorsal side of the patagium. Most specimens of *A. rhinopomatos* were situated on the ventral side of the patagium near the side of the body, inside the hairy area, and some were found on the venter. Only three specimens were found on the shoulder, and another on the back. Nearly all were found with their posterior end towards the tail of the bat, so that it pointed upwards when the bat rested.

The insects were all lying in the subcutaneous tissue, and macroscopically there was no indication of inflammation visible near specimens embedded in the patagium, where the blood vessels could be observed clearly under the binocular microscope. Sections through the insect embedded in the skin of the bat showed the formation around the insect of a fibrous capsule, the thickness of which varied in different specimens. This fibrous capsule was pulled into the funnel formed by the anterior part of the abdomen of the insect, forming a long, conical process. Beyond the unbroken fibrous capsule there was an area of oedematous tissue consisting mainly of elastic fibres and remnants of cells. In some cases normal capillaries were present in the area and there were indications of mild early inflammation. In other cases it was apparent that there had previously been acute inflammation which had nearly completely receded, leaving a light, chronic inflammation. No signs of severing of blood vessels or of extravasation of blood were observed. On the other hand, the hind-gut of the *Ascodipteron* and the faeces contained digested blood as proved by application of the benzidine test to the faeces. The amount of faeces produced was very small. It appears, therefore, that during the process of embedding the insect causes initial irritation and inflammation which disappears eventually, and that the embedded female causes remarkably little reaction in the tissues of the host. The insect causes the subcutaneous tissue in front of its mouthpart to disintegrate by continuous sucking action, and apparently ingests mainly serous fluid. The blood ingested is probably obtained by extravasation from the regional capillaries, and this is rapidly cleared away by suction. The absence of a stronger tissue reaction than the slight changes described suggests that the changes are due to mechanical action and not to the injection of an irritating saliva.

DESCRIPTION. The embedded female of *A. rhinopomatos* has been described by Jobling in 1952 (Figs. 94–95). Its main characteristics are as follows: length 4·5 mm., width 2 mm.

The insect is much more weakly chitinized than *A. africanum*, and the peg-like spines on the theca of the labium and on the pleurae are less pigmented. The theca of the labium is conical, viewed dorsally and laterally, 0·4 mm. long and 0·3 mm. broad at the base. The peg-like spines cover a triangular area on the surface of the theca with the base of the triangle laterally. There are 4 to 5 rows of these pegs, which are small and unpigmented. The teeth of the 4 median dorsal rows of the labella are very thin and curved in an S-shape with secondary serrations. The teeth of the lateral dorsal rows are long, hooked, also with secondary serrations. The

teeth of the lateral and ventral rows are sharp, falciform blades. The pedicel of the antennae bears a single short seta dorsally in all specimens examined. The presence of two setae as figured by Jobling (1952) is probably accidental. Gena covered with

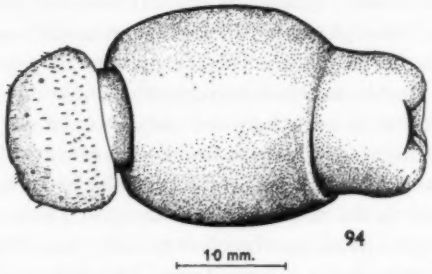



Fig. 94. *Ascodipteron rhinopomatos*, embedded female.

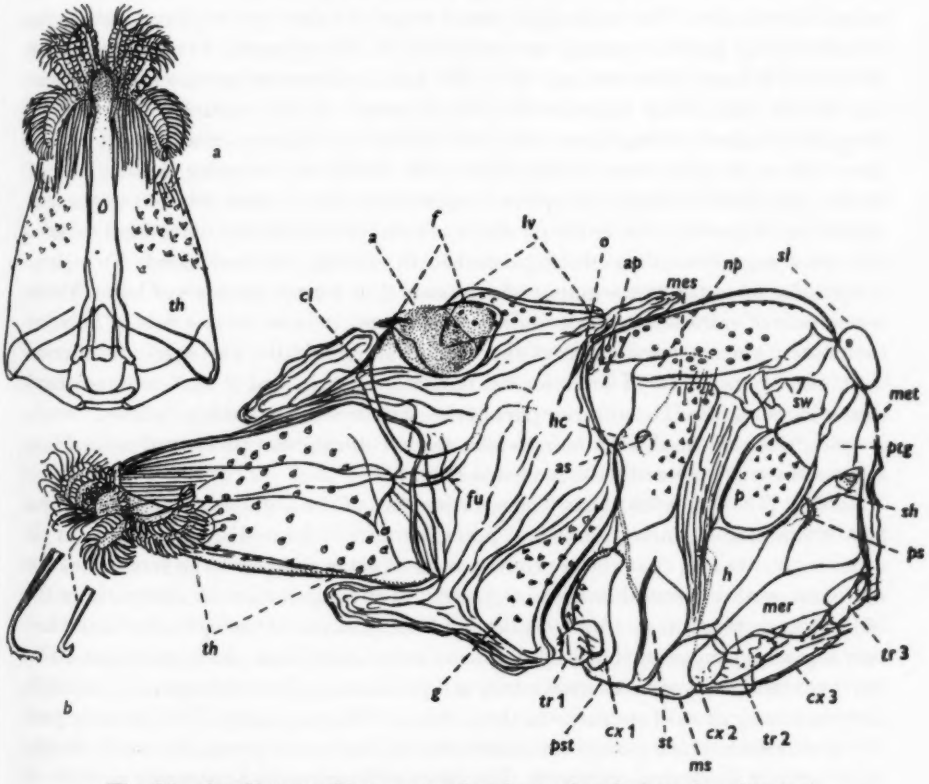



Fig. 95. *Ascodipteron rhinopomatos*, female: *a*, dorsal view of proboscis; *b*, lateral view of head and thorax (after Jobling, 1952).

peg-like spines except at its three corners. Upper part narrow and curved, anterior corner opposite middle of fore coxa, which bears a few pegs or hairs near its anterior border. Scutum roughly octagonal, parallel-sided. Jobling states that

'the prescuto-scutum is broader in the anterior part than in the middle'. In some specimens examined there is little difference between the width of the scutum anteriorly and in the middle, and in some specimens it is even slightly narrower anteriorly than in the middle. Scutellum broadly rounded, with a single seta near each lateral margin. Mesopleura with slightly rounded, postero-dorsal corner which bears a few fine setae. Below these a number of pegs along the posterior margin. In the two freshly hatched females the whole dorsal half of the mesopleura is covered with setae and there are only 8 or 10 pegs in its ventral half. Pteropleuron with straight dorsal and rounded posteroventral margin, covered with pegs in some specimens, but covered with hairs in its dorsal half with a few pegs in its ventral part in the newly hatched females. The occurrence of pegs and hairs and their arrangement seems therefore to vary to a considerable extent.

Dorsal abdominal spiracles in a curved row. The distance between the ventral spiracles, which lie at the sides of the genital opening, less than that between the lateral dorsal ones. The sclerotized raised rings of other species (cerci) which lie dorsally to the genital opening are reduced in *A. rhinopomatos* to small, irregular plates which bear either two, one or no fine hairs and several minute spines. There is a double row of fine hairs in the area between the six spiracles and another irregular row surrounding them. Anterior to this row there is a row of short spines, then follows another row of fine hairs, and finally an irregular double row of spines. This order of hairs and spines is apparently due to their relation to the last abdominal segments, the limits of which are obliterated in the embedded female, but these segments can be distinguished in the freshly hatched insect. Two pairs of spiracles lie in the last segment which bears 2 to 3 irregular rows of hairs. There is one pair of spiracles in the penultimate segment, which bears a row of hairs at the posterior margin and a row of short spines in its middle. The segment anterior to it also bears a row of hairs at the posterior margin and 2 to 3 rows of short spines in its middle. The anterior part of the abdomen of the freshly hatched female is cylindrical, covered with hairs which fall off during the process of embedding and no trace of segmentation is visible (Fig. 96).

Jobling (1952) suggests that 'the anterior part of the abdomen is formed by the first abdominal segment, while the posterior part is formed by the fusion of all other segments and that the concentric rows of setae represent the remains of the marginal setae of these abdominal segments'. The arrangement of alternating rows of hairs and spines in *A. rhinopomatos*, and the position of the spiracles in the last two segments, suggests that each segment bears more than one row of hairs. The two proximal rows of hairs apparently are the marginal rows of segments 4 and 5, and the row or rows of spines lie in the middle of these segments. The posterior part of the abdomen which carries concentric rows of hairs thus seems to consist of only three or four abdominal segments. The last visible segment apparently consists of two fused segments, as it has two pairs of spiracles. The spiracles may, however, have been displaced so much posteriorly that the original relation to a particular segment cannot be deduced with certainty from their present position. The position of the spiracles in the young female suggests that the two pairs of spiracles in the last segment, that is the spiracles of segments 6 and 7 according to Jobling,

are the median dorsal and ventral spiracles of the embedded female. The spiracles of the penultimate segment, the 5th segment according to Jobling, are the lateral dorsal spiracles of the embedded female.

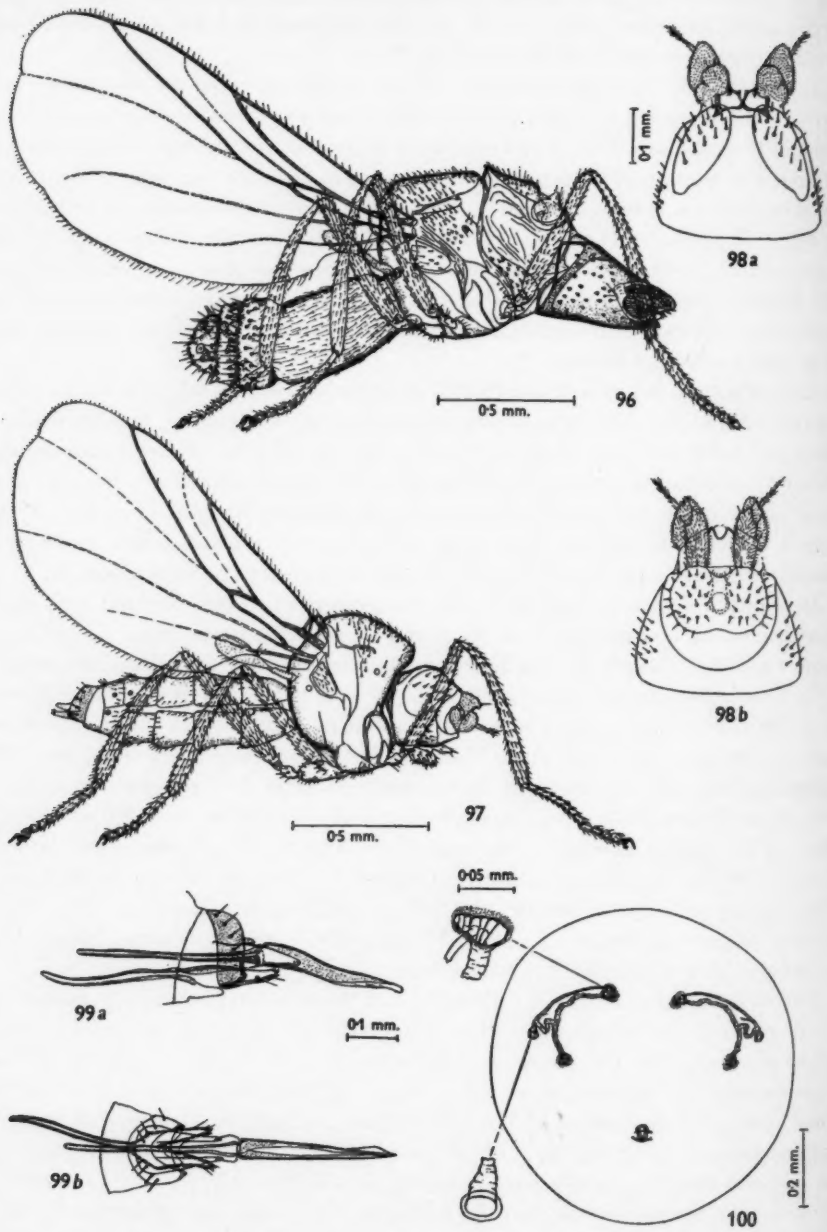
The legs of the young female (Fig. 96) are slender, covered all over with short hairs. The femora are of about equal length, while the fore tibiae are longer than the middle and hind tibiae. The wings differ somewhat from those of *A. africanum*. Length 1·65 mm., width 0·8 mm. The indentation near the junction of $M_{1+2}$ with the wing margin is more marked and there is a similar indentation near the end of $R_{4+5}$. $R_{2+3}$ reaches the costa. There are only a few scattered short setae (1 to 3) on $R_1$ and $R_{4+5}$, while these veins have a continuous row of setae in *A. africanum*. The halteres are long, club-shaped and bear a few hairs at the ventral side of the thick part and some hairs dorsally nearer the base. They were found to be present in several embedded females.

*Male* (Figs. 97, 98). Similar to that of *A. africanum* in general. It is very weakly sclerotized and its wings are smaller than those of the female. Length without aedeagus 1·4 mm., wing length 1·4 mm., width 0·7 mm. Head quadrangular dorsally, as broad as mesonotum. The elliptical latero-vertices are covered with small hairs in the dorsal half. Genae covered with similar hairs (Fig. 98). Palps narrow, tapering to a point which bears a short seta. Antennae similar to those of female, protruding in front of head. Theca of labium transversely elliptical.

Mesonotum similar to that of female but narrower in front, covered with short hairs except for a narrow median stripe and a transverse stripe along the posterior border in which there is a single hair in each lateral corner. Scutellum rectangular with rounded posterior border and a single seta near each side. Laterally the shape of the thorax resembles that of the female, except that the limits of the sclerites are less clearly marked. Thus, the mesopleuron appears fused with the antepronotum, and it seems also fused ventrally with the sternopleuron without any visible demarcation. Both mesopleuron and pteropleuron bear hairs only and the peg-like spines present in the female are absent. Wings similar to those of female. In life they are held fully extended horizontally above the abdomen. Legs similar to those of female. All femora of about equal length. Front tibia slightly longer than femur. Middle and hind tibiae as long as femora. Hind tibiae markedly thicker distally than at the base.

The abdomen of the male is cylindrical and the segments are clearly marked by constrictions in the living insect. There are rows of hairs along the posterior margins of the segments. Only three pairs of spiracles are present, two of them in the last segment and one pair in the preceding segment. They are provided with rods of their closing mechanism as in the female, but the rods are shorter and have an angular bend. This arrangement of the spiracles in the last abdominal segments has an obvious function in the female, where this is the only part of the body in contact with the outside air, but the presence of a similar arrangement in the free-living male is remarkable.

It is interesting to note in this connexion that there is a similar arrangement in the female of *Tunga penetrans* which also lives embedded in the skin. The spiracle of the first abdominal segment is normal, resembling that of other free-living fleas.




Figs. 96–100. *Ascodipteron rhinopomatos*. 96, freshly hatched female, profile; 97, male, profile; 98, male, head: *a*, dorsal; *b*, ventral; 99, male genitalia: *a*, profile; *b*, ventral; 100, puparium, spiracles.

It does not function in the embedded female as it is closed by the tissues of the host. The spiracles of segments 2 to 4 are vestigial. The spiracles of segments 5 to 8 are enlarged and concentrated in the posterior part of the body which alone is in contact with the outside air. In the free-living male, however, spiracles are present in all 8 abdominal segments as in other fleas.

*Genitalia* (Fig. 99). The hypopygium consists of a ring which is more heavily sclerotized than the rest of the abdomen and bears a row of about 10 hairs. The genital apparatus resembles that of *Nycteribosca* in general outline and is retracted wholly inside the body in the resting position. The aedeagus is a tapering tube, membranous ventrally and more heavily sclerotized dorsally. At its base it is articulated with a rod-like apodeme which is slightly longer than the aedeagus. There is a wide aedeagus sheath with a long, slightly curved apodeme. The aedeagus sheath ends posteriorly in two processes which bear 2 to 3 short, curved spines and a hair at the tip. Another hair stands further basally. These processes may be considered as the parameres in analogy to the position in other genera. In addition there are two bare processes dorsal and lateral to the aedeagus sheath which are considered as claspers by Jobling (1940).

*Puparium* (Fig. 100). The puparium is of light brown colour with a darker band around its middle. It is about 1·5 mm. long and 1 mm. wide in the female and slightly smaller in the male. The puparium is so transparent that the pupa is clearly visible and its sex can be determined by the differently shaped head. The puparium is ovoid in shape and there are four spiracles at its posterior end and two more which are not functional. There is a rounded lid anteriorly which reaches from the frons of the pupa to about the middle of the thorax, so that pressure of the emerging insect on the anterior rim opens it from in front backwards. The puparium was found generally near the female in the hairs of the bat, which form a kind of funnel around the upward-pointing posterior end of the insect, but it falls to the ground as soon as the bat moves.

## V. HEMIPTERA

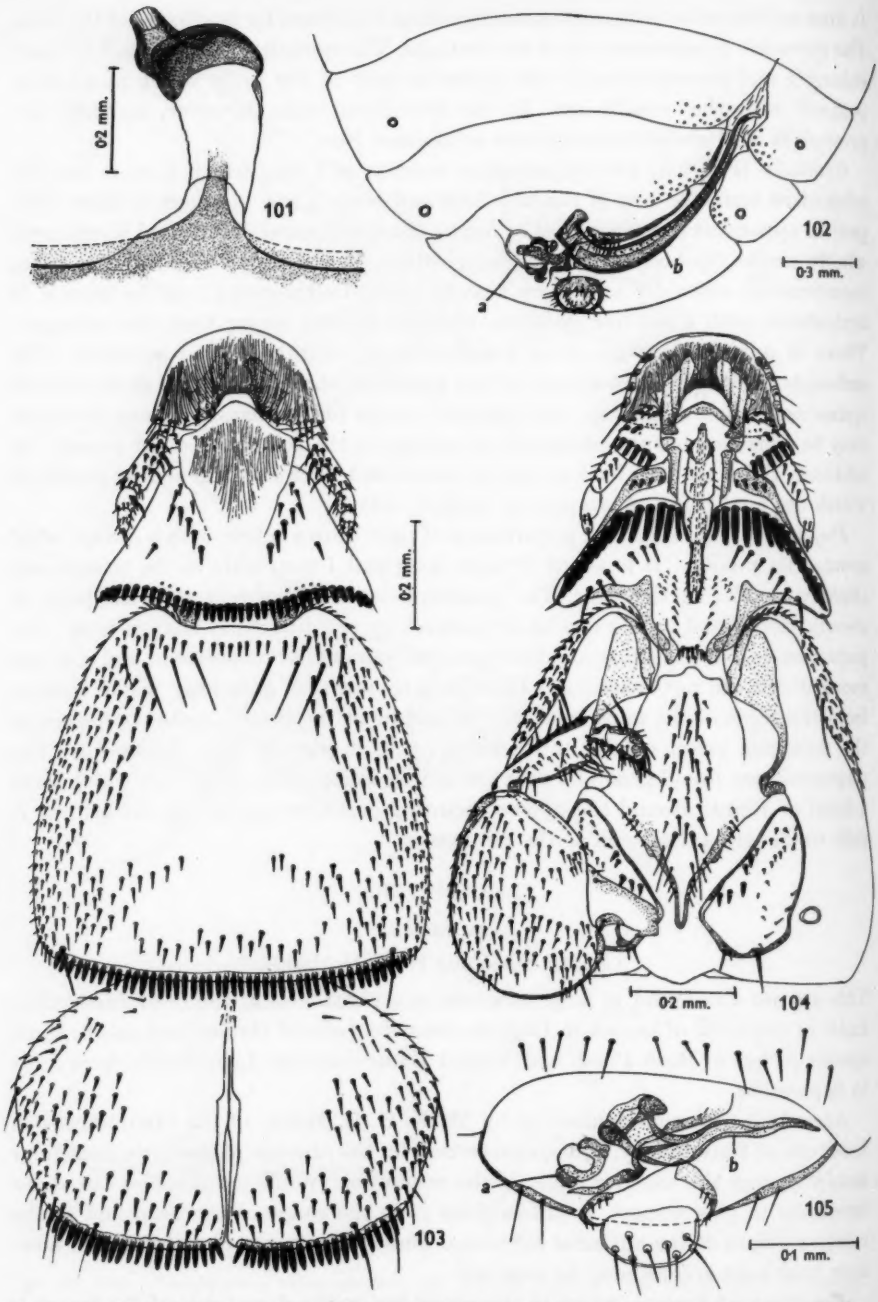
### Cimicidae

### *Cacodmus vicinus* Horvath, 1932

This species was found in large numbers as a parasite of a colony of *Pipistrellus kuhli* in the roofs of houses in Dagania near the Lake of Galilee, and on the same species of bat in Rosh Pinah and Yessod Hamaalah near Lake Huleh, from June to September.

According to a communication by Mr N. C. E. Miller, of the Commonwealth Institute of Entomology, it is characterized by the absence of the little triangular sinuosity near the anterior angle of the pronotum. While it resembles *Cacodmus tunetanus* in this respect, it differs from it in the shape of the head and in the relative length of the antennal segments. These differences are rather small, however, and both species may be identical.

The organ of Berlese, which in this genus lies on the dorsal side of the insect, is of rather characteristic shape as shown in Fig. 101, and a figure of the male genitalia is given (Fig. 102).




Figs. 101, 102. *Cacodmus vicinus*. 101, female, Berlese's organ; 102, male genitalia: *a*, phallosome; *b*, 'penis'. Figs. 103–105. *Eoctenes intermedius*. 103, female, head and thorax, dorsal; 104, female, head and prothorax, ventral; 105, male, genitalia: *a*, phallosome; *b*, 'penis'.

*C. vicinus* has been described from Tunisia from *Pipistrellus kuhli* and is thus probably widely distributed in the Southern Mediterranean.

### Polyctenidae

*Eoctenes intermedius* Speiser, 1904

A male and a female of these rare insects were found on *Taphozous nudiventris* in Tel Aviv in August 1946. The species was originally described from Egypt from *T. perforatus* and was later found in the Sudan. This is thus another example of the occurrence of Ethiopian elements in the Middle East.

Description. Length: male 2·5 mm., female 3 mm.

*Head.* Labrum semicircular with a row of small setae posteriorly. Head triangular, with sharp, posterior corners and with 2 rows of short dorsal setae which converge posteriorly along the raised central part of the head, and a single seta in the lateral part. A ctenidium of 36 short spines at the posterior margin. On the ventral side a curved row of about 16 spines on each side of the head and a row of short spines in front of it. Proboscis consisting of 4 segments. Antennae not reaching posterior angles of head, with broad basal segment which bears a row of 5 blunt short spines ventrally along its posterior border and some curved short spines anteriorly.

*Thorax* (Figs. 103, 104). Pronotum as long as broad, narrower anteriorly, covered with short spines laterally, leaving a bare median stripe. A row of short spines along anterior margin, and behind it a row of 6 to 8 longer hairs. A double row of short spines before the hind margin and a similar row, which is broadly interrupted in the middle, further anteriorly. A longer seta in each posterior lateral corner. A ctenidium of 40 blunt spines at the posterior margin. Elytra rounded laterally, incompletely divided in middle, with ctenidium of 18 short, blunt spines at the posterior margin in each half, covered with short spines in the posterior and lateral half and with thinner hairs in the anterior lateral part. Two longer setae in each posterior lateral angle. Prosternum long, with pointed process between the fore-coxae. Claws markedly asymmetrical in middle and hind legs.

Abdominal segments with single row of spines in middle in the male, and double or triple row in the 4 posterior segments in the female. There are 2 to 3 longer setae on segments 6 and 7 in the female.

Genitalia of male, see Fig. 105.

## VI. SIPHONAPTERA

A most surprising feature was the extreme scarcity of fleas as compared with other bat parasites. Only eight specimens were found on the hundreds of bats examined. All belong to well-known species.

*Ischnopsyllus consimilis* Wahlgren, on *Pipistrellus kuhli*. Rosh Pinah, 24 April 1946, 1 ♀. Dagania, 26 June 1944, 4 ♀, 1 ♂.

*Rhinolophopsylla unipectinata* Tasch., Jerusalem, 1 ♀.

*Leptopsylla segnis* Schönh. *Pipistrellus kuhli*, 1 ♂.

This species, as far as is known, has not been recorded previously from a bat.

## VII. DISTRIBUTION OF BAT PARASITES

Of the seventeen species of Nycteribiidae recorded from the Western Palaearctic region, twelve were found to occur in Palestine. Only *P. monoceros* Speiser, *Basilia nattereri* Kol. and *Nycteribia kolenatii* n.nom. have not so far been found outside Europe. The status of *E. aegyptia* Macq. could not be cleared up as no specimens were available for study. Of the twelve species found in Palestine, two are so far known only from the Middle East (*Nycteribia biloba* n.sp. and *N. integra* n.nom.). *Eucampsipoda hyrtlii* belongs to the Ethiopian fauna and enters the Palaearctic region in the Middle East with the only Megachiropteron in the area. Its northern limit of distribution is in Antioch in southern Turkey. It has been recorded previously from tropical and South Africa and from the Oriental region, but, as pointed out above, is known with certainty only from Arabia, Egypt, Palestine and Syria, and probably occurs in East Africa. It will also probably be found in Cyprus where *Rousettus aegyptiacus* occurs. All material from the Oriental region and South Africa examined belongs to other species. Some records from tropical Africa also probably refer to other species and need confirmation. Its southern limit of distribution in East Africa is not precisely known.

*Basilia bathybothyra* was originally described from India and later recorded from Africa. The occurrence of a subspecies in Palestine and Cyprus has been recorded above. Examination of specimens from India and Africa has shown that the Mediterranean subspecies is most clearly related to the Ceylon form, and that there are at least two other forms in Africa and Indonesia. The route of spread of this species seems therefore to go through the Middle East. In other groups of Diptera (Anopheles, Phlebotomus) there is evidence that the species originated in Africa and spread through the Middle East into Western Asia. The origin of *B. bathybothyra* is more probably in the Oriental region, where the main centre of the family is situated.

Apart from the above species, the nycteribiid fauna of the Middle East is thus mainly palaearctic. This is to be expected, as most bats occurring in the Middle East also have a wide distribution in Europe and Asia, and only five species belong to the Ethiopian fauna.

Through the kindness of Dr R. Wenzel of the Chicago Natural History Museum a large collection of Nycteribiidae from the Yemen and Egypt, collected by Dr H. Hoogstraal, was examined. The collection from the Yemen consisted mainly of *Nycteribia integra* (85 specimens), *Cyclopodia greeffi arabica* n.subsp. (14 specimens) and *Penicillidia fulvida* Bigot (1 specimen). The latter two species belong to the Ethiopian fauna, while as far as is known, *Nycteribia integra* is a species endemic in the Middle East. The collection from Egypt contained only three species: *Eucampsipoda hyrtlii* (223 specimens), *Nycteribia integra* (89 specimens) and *N. biarticulata* (118 specimens). Except for two specimens of *N. integra*, all specimens of the latter species were found on species of *Rhinolophus*. No Nycteribiidae were found, as Dr Hoogstraal informs me, on hundreds of specimens of *Pipistrellus kuhli*, *Taphozous perforatus* and *T. nudiventris*. Some specimens of *Basilia bathybothyra* from Egypt were examined which belong to the new subspecies described in this paper.

## Table 1. *Distribution of parasites on various species of bats*

C. Index Catalogue, Stiles; F. Falcoz; K. Karaman; Ko. Kolenati; J. Jobling; S. Speiser; Sm. Smart; St. Stefanelli. Heavy type indicates that this is the only or the chief host of the parasite in Palestine.

| Species of bat | *Rousettus aegyptiacus* | *Rhinolophus ferrum-equinum* | *R. hipposideros minimus* | *R. euryale* | *R. blasii* | *Myotis myotis* | (?) *M. mystacinus* | *M. nattereri* | *Miniopterus schreibersi* | *Pipistrellus kuhli* | *Rhinopoma microphyllum* *R. hardwickei* | *Asellia tridens* | *Taphozous nudiventris* | *Tadarida teniotis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **STREBLIDAE** | | | | | | | | | | | | | | |
| *Nycteribosca kollari* | — | **22** C.F.J.St. | 6 C.F.J. | 4 C.F.J.St. | 12 C.F.J. | — C.F. | — | — | 1 C.T.J.St. | — | — C.J. | — C.J. | — | — |
| *N. alluaudi* | — | — | — | — | — | — | — | — | — | — | **85** | — | — | — |
| *Raymondia setosa* | — | — | — | — | — | — | — | — | — | — | — | 1 | — | — |
| *Ascodipteron rhinopomatos* | — | — | — | — | — | — | — | — | — | — | **23** | — | — | — |
| **NYCTERIIDAE** | | | | | | | | | | | | | | |
| *Nycteribia pedicularia* | 1 | — | — S.F.C. | — | — | **41** K.F.S.C. | 29 S.F. | 23 | 8 S.F.C. | — | — | — | — | 1 |
| *N. latreillii* | — | — K. | — | — K.St. | — | **21** K.C.St. | — | — | — K. | — | — | — | — | — |
| *N. schmidlii* | 5 | — K.F.St. | — K. | — K.St. | — | 7 K.S.F.C.St. | — S.F. | — | **77** K.S.F.C.St. | — | — | 1 | — | — |
| *N. vexata* | — | — K.F.C. | 1 F.C.S. | — K.F.C. | — | **20** K.F.C.St. | — | — | — F.S. | — | — | — | — | — |
| *N. biarticulata* | — | 3 K.S.F. C.Sm.St. | 3 S.F.C. Sm.St. | 9 K.S.F. C.St. | 7 F.C. | 5 S.F.C. | — | — | 1 C. | — | — | — | — | — |
| *N. biloba* | — | 1 | 5 | — | — | 1? | — | — | 2 | — | — | — | — | — |
| *N. integra* | — | 1 | (1 on *Rhinolophus* sp.) | | | — | — | — | — | — | — | — | — | — |
| *Basilia nana* | — | — | — | — | — | 8 S.C. | — S.C. | **122** S.C. | — | — | — | — | — | — |
| *B. bathybothyra daganiae* | — | — | — | — | — | — | — | — | — | **10** | — | — | — | — |
| *Penicillidia dufourii* | — | 1 K.S.F.C. | 1 S.F.C. | — F.C. | — S.F.C. | 63 K.S.F.C.St. | 30 | 31 | 85 K.S.C.St. | — | — | 1 | — | — |
| *P. conspicua* | — | — | — K. | — S.F.C. | — S.F.C. | 7 S.F.C.St. | 10 | — | 46 K.S.C.F. | — | — | — | — | — |
| *Eucampsipoda hyrtlii* | **176** S.Ko. | — | — | — | — | — | — | — | — | — | — | — | — | — |
| **HEMIPTERA** | | | | | | | | | | | | | | |
| *Cacodmus vicinus* | — | — | — | — | — | — | — | — | — | **20** C. | — | — | — | — |
| *Eoctenes intermedius* | — | — | — | — | — | — | — | — | — | — | — | — | 2 | — |
| **SIPHONAPTERA** | | | | | | | | | | | | | | |
| *Ischnopsyllus consimilis* | — | — | — | — | — | — | — | — | — | 6 | — | — | — | — |
| *Rhinolophopsylla unipectinata* | — | 1 'on bat' | — | — | — | — | — | — | — | — | — | — | — | — |
| *Leptopsylla segnis* | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — |

The small number of species of Nycteribiidae occurring in Egypt is apparently due to the absence of bats of the genera *Myotis* and *Miniopterus* in this country. In Palestine these two genera are parasitized by nine species of Nycteribiidae and are the chief hosts of the species of *Penicillidia* and of the subgenus *Nycteribia*. It is surprising, however, that no more Ethiopian species of Nycteribiidae have been found on the many Ethiopian species of bats occurring in Egypt.

Only very few species of nycteribiids occur in the Ethiopian as well as in the palaearctic region. In addition to *Eucampsipoda hyrtlii*, *Nycteribia schmidlii* occurs throughout Europe and Africa. The distribution of *N. schmidlii* in Africa, however, needs further study, and the species may eventually have to be split up into subspecies.

Many of the European species of nycteribiids extend eastwards into Asia in accord with the wide distribution of some species of bats in Europe and Asia. Records of European species of Nycteribiidae from East Asia, however, have to be accepted with caution. *Penicillidia dufourii* has been recorded from Europe, China and Formosa. Karaman (1939) showed that the specimens from Formosa belong to another species, *P. tainani*. Examination of Chinese material from the collection of the British Museum has shown that these specimens also belong to *P. tainani*. A specimen from the Himalayas, also from the collection of the British Museum, belongs, however, to *P. dufourii*. The eastern limit of distribution of this species is thus not exactly known. Similarly, examination of specimens identified as *Nycteribia pedicularia* from China has shown that they are in fact *N. formosana* Kar. and the record of *N. pedicularia* from South Africa refers to *N. capensis* Kar.

## VIII. HOST-PARASITE SPECIFICITY

Table 1 gives the distribution of parasites on the various species of bats. Figures for the numbers of parasites per bat could not be given, as a large part of the material was collected without noting the number of bats examined. The figures of the numbers of parasites thus are intended to give only an approximate picture of their frequency. The letters indicate the names of authors who have recorded the parasite for the particular host. Some of these records are probably incorrect as some species have not been previously separated (*Basilia nattereri* and *B. nana* and particularly *Nycteribia pedicularia*, *N. latreillii* and *N. kolenatii*).

There seems to be a true host-parasite specificity in a few cases only. Thus *Eucampsipoda hyrtlii* was found only on *Rousettus aegyptiacus*, except for a single specimen on *Rhinopoma hardwickei cystops*. *Basilia bathybothyra daganiae* was found only on *Pipistrellus kuhli*. *Nycteribosca alluaudi* and *Ascodipteron rhinopomatos* were found only on *Rhinopoma microphyllym* and *R. hardwickei arabium*.

Some other species were found chiefly on a single species of bat with occasionally a few specimens on other bats. Thus, *Nycteribia latreillii* was found in Palestine only on *Myotis myotis*. Karaman records this species also mainly from *M. myotis* (70 specimens) and only three specimens from *Rhinolophus ferrumequinum* from Yugoslavia and from *Myotis myotis*, *Rhinolophus euryale* and *Miniopterus schreibersi* from North Africa. Similarly, *Basilia nana* was found in Palestine mainly on *Myotis nattereri* and only a few specimens on *M. myotis*. *Nycteribia*

*schmidlii* was found in Palestine mainly on *Miniopterus schreibersi* and only a few specimens on *Myotis myotis* and *Rousettus*. Karaman in Yugoslavia found *Nycteribia schmidlii* also mainly on *Miniopterus schreibersi* and only a few specimens on *Rhinolophus euryale, R. ferrumequinum* and *R. hipposideros*, and mentions also *Myotis myotis* and *B. barbastella* as hosts. The same author also gives records of some specimens of *Nycteribia schmidlii* from Tunisia on *Miniopterus schreibersi* and *Myotis myotis*.

In other cases parasites seem to be restricted to a family of bats. Thus, *Nycteribosca kollari* was found in Palestine only on the four species of *Rhinolophus* and only a single specimen on *Miniopterus schreibersi*. *Penicillidia dufourii* and *P. conspicua* were found only on the various species of *Myotis* and on *Miniopterus schreibersi*, but other authors record both species from various species of *Rhinolophus* as well, as shown in the table. It seems, therefore, that in these cases there is no true specificity, but that the restriction to a group of bats is probably due to their roosting habits, which apparently differ in various countries. *Nycteribia pedicularia* has also been found in Palestine only on the various species of *Myotis* and on *Miniopterus schreibersi*. Records from other species of bats will have to be checked as this species has not been separated in the past from *Nycteribia latreillii* and *N. kolenatii*. *N. vexata* has been found in Palestine only on *Myotis myotis*. This species, however, is not host specific at all, as the records of other authors show. *Nycteribia biarticulata* has been found on various species of *Rhinolophus, Myotis myotis* and *Miniopterus schreibersi* without marked preference for any particular species.

The observations given above on the whole confirm those of Stefanelli (1942) on Nycteribiidae and Streblidae in Italy. This author found *Nycteribosca africana* (*N. kollari*) mainly on *Rhinolophus euryale* and showed by experiments that *Nycteribosca africana* showed a definite preference for this species of bat. He found *Nycteribia biarticulata* to be restricted to the genus *Rhinolophus*. *Penicillidia dufourii* was found mainly on *Myotis myotis* and a few specimens only on *Miniopterus schreibersi*. *Nycteribia schmidlii* was found mainly on *Miniopterus schreibersi* and only a few specimens on various other bats. *Nycteribia vexata* was found only on *Myotis myotis* except for a single specimen on *Rhinolophus ferrumequinum*.

Stefanelli's figures for *Nycteribia pedicularia* were checked by re-examination of his specimens. Only those found on *M. capaccinii* proved to be *Nycteribia pediculata*, while all those found on *Myotis myotis* were *Nycteribia latreillii*. The specimens identified by Stefanelli as *N. blasii* also proved to be *N. latreillii*.

This restriction to a group of bats in a certain area is well illustrated by the difference between the nycteribiid fauna of Palestine and Egypt described above. None of the species of *Basilia, Penicillidia* and of the subgenera *Nycteribia* and *Acrocholidia* which occur in Palestine have been found in Egypt. This is apparently due to the absence in this country of bats of the genus *Myotis* and *Miniopterus*, which are the main hosts of these Nycteribiidae in Palestine. There were no specimens of *Nycteribia vexata* in the material from Egypt, and a record of this species from this country found in the literature will have to await confirmation by new material.

The number of species of parasites which were found on a species of bat vary in a similar manner. Eight species of Nycteribiidae were found in Palestine on

*Myotis myotis*, that is, all except the quite specialized species and those of which so few specimens were found that nothing definite can be said about them. Seven species of parasites were found on *Miniopterus schreibersi*. Only two to four species of parasites were found on the various species of *Rhinolophus* in Palestine, but many more are recorded from other countries. This again may be due to their roosting habits.

*Pipistrellus kuhli* in Palestine has a quite specialized parasite fauna. It consists of only one species of nycteribiid, (*Basilia bathybothyra*), not found on other bats, and of *Cacodmus vicinus* and *Ischnopsyllus consimilis*. This is almost certainly due to the fact that *Pipistrellus kuhli* lives in an environment quite different from that of other bats in Palestine. It does not enter caves, but is found mainly in the roofs of houses. There may, however, exist a specific host-parasite relationship between *Basilia bathybothyra* and the genus *Pipistrellus*, as several specimens of this species from India were also found on species of *Pipistrellus*.

The Ethiopian species of bats have parasites peculiar to them. The occurrence of *Eucampsipoda hyrtlii* on *Rousettus* alone has been noted. Only two species of Streblidae (*Ascodipteron rhinopomatos* and *Nycteribosca alluaudi*) were found in considerable numbers on *Rhinopoma microphyllum*. The other parasites (*Eoctenes intermedius* on *Taphozous nudiventris* and *Raymondia setosa* on *Asellia tridens*) were found in such small numbers that no conclusions can be drawn.

There seems thus to be a true host-parasite specificity in a number of cases either between species or between groups of species, but in other cases restriction of a parasite to one species or to a group of species seems to be due more to the habits of bats in a given area than to true specificity.

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# The Journal of General Microbiology

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